EFFECT ON SOCIO-ECONOMY AND BIO-ECOLOGICAL RELATION OF YARTSA GUMBA AN ENTOMO-FUNGAL COMPLEX IN PITHORAGARH HIMALAYA

ABSTRACT

THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

ZOOLOGY

By

SHAHID SAMI SIDDIQUE

DEPARTMENT OF ZOOLOGY
FACULTY OF LIFE SCIENCES
ALIGARH MUSLIM UNIVERSITY
ALIGARH - 202002 (INDIA)

2006

ABSTRACT

Yartsa gumba or dbyar-rtswa-dgun-bu is Tibetan name of an Entomo-Fungal combination between *Hepialus armoricanus* (Oberthur). (Lepidoptera; Hepialidae) larva and its parasitizing fungus *Cordyceps sinensis* (Berk), which is traditionally used in Tibetan and Chinese System of Medicine (TCM). This medicinal entomo-fungal product is known in Chinese as Dong Chong Xia Cao (winter worm and summer plant or grass in summer and worm in winter), Yartsa Goenbub in Bhutan, Yarchagumba means herbs of life in Nepal and Tochukaso in Japan. It is also known as Caterpillar mushroom, Caterpillar fungus, vegetable wasp, plant worm, vegetable caterpillar (Cooke, 1892) and in India it is commonly known as Keera Ghas. This entomo-fungal combination is known to be used for many centuries as tonic, medicine and aphrodisiac and in religious ceremonies in China, Indonesia and Upper Himalayas. The medicinal importance of this combination is due to a fungus viz., *C. sinensis* parasitizing the host caterpillars i.e. *H. armoricanus* (Arif & Kumar, 2003).

The objective of the present study was to study the bio-ecology of Yartsa gumba (Entomo-fungal), its mass rearing and socio-economic impact. To carry out the study four expeditions were taken to four different high altitude areas ranging from 10000-14500 feet altitude from 2004-2006, Larval and pupal stages were collected from different

locations and kept inside the tentage accommodation for the study of the life cycle. Some of the specimens were carried to lower altitude to rear them in laboratory condition, however, none of them survived because of high temperature. The number, population density, habitat, length of uninfected and infected larval stages, stromata and weight were recorded in fresh as well as in dried condition. It was recorded that specimens are usually with one and two stalks from head region, however, unusually three specimens were recorded to have four stalks i.e two stalks each from head and caudal region.

For mass multiplication of Yartsa gumba spores and tissues were taken in eight different culture media in asceptic condition in high altitude areas as well as in laboratory condition. The culture media used were Potato Dextrose Agar (PDA), Casein Hydrolysate Dextrose Agar (CHDA), Beef Extract Dextrose Agar (BEDA), Soyabean Extract Dextrose Agar (SEDA), Rice Extract Dextrose Agar (REDA) and Black Soyabean Extract Dextrose Agar (BSEDA), It was observed that the tissue taken from the stromal region of the fungus is the most suitable for inoculum to get the mycelia run in the culture. However, spores and stalk tissue did not response at all. Out of the 8 culture media the mycelial growth was successful on 5 culture media viz., PDA, BEDA, CEDA, SEDA and REDA .During the experiment it was also observed that the optimum growth of the fungus occurred under

low temperature condition between 5 to 15^0 C and more acidic pH (5-5.5). However, sclerosis was observed in the mycelium obtained on all the types of the culture media.

During the study of Yartsa gumba families of 24 villagers depending on Yartsa gumba were contacted to share their experiences, and views for correct assessment to their livelihood and socio economic status and sources of their income. Total population of 24 villages is 2363 out of which male and female are 1231 (52.09%) and 1132 (47.90%) respectively. Further out of these, schedule tribes and schedule caste are 408 (17.27%) and 1000 (42.32%) respectively. During investigation it was noticed that entire schedule tribes and schedule caste population is 1408 of the total population engaged in the collection of Yartsa gumba as an alternate source of income in short summer period. Main source of income of the inhabitant is cultivation of vegetables, rajmash in their fragmented small holdings in hilly terrain, maintenance of small herd of sheep and goat for wool and meat production and the newly inducted German Angora rabbit for wool production. Some people also use to visit high altitude areas to collect medicinal and herbal plants for argumentation of their revenue generation. The poor income by their small holding and other sources force the local people to migrate to plain areas in search of jobs and

this has been practiced for a long time. However, the discovery of Yartsa gumba a high medicinal value natural resource in recent past has opened a new avenue for the unemployed youths.

Migration in high altitude along with their herd is also a practice of local people where they use to grow some short duration vegetable crops and during onset of winter they further migrate down to their main habitat. Thus the locals earn their livelihood from different sources. In present scenario during May to mid July locals go to the areas of natural habitat of Yartsa gumba and keep a vigil on the appearance of Yartsa gumba and start collection. This short term collection of Yartsa gumba is more paying as compared to their traditional year long agri-horticulture and animal venture besides, fabrication of wool products like, shawl, tholma, carpet, dun, punkhi *etc.* Moreover, market of Yartsa gumba is very fast and there is no delay in disposal of high value medicinal raw material as compared to their traditional products.

It has been observed that Yartsa gumba being sporadic and scattered in nature is not easily visible to the eyes. Local sits on the grounds and sometime they lie on the ground and spot out stalk which is more or less green grass blade like structure and almost camouflage with the natural growing grass of the area, however, its peculiar

structure differs. Local people dig out the specimen keeping in view to avoid breakage of stalk and put it in aluminum plates or baskets. It has been recorded that after rigorous collection, one skill person can collect 30-40 specimen of Yartsa gumba per day, however, quantity of collection also depends on the locations, occurrence and abundance of the specimen. Collection of Yartsa gumba has opened a new avenue for the revenue generation of the locals and improvement in their socio economic condition. It was observed that during digging a large number of uninfected larval/pupal stages arc either exposed or damaged because of ignorance of gatherers. However, a few of them are aware and they further keep such stages safely under the soil and show keen interest to conserve it for further harvesting. In the present study it was observed that a skill person collecting 30-40 specimen earn about Rs.400-500 per day after strenuous efforts without wasting his time. Thus in one season of 60 days Rs. 17000-25000 can be earned by one skill person. However, one family consisting of 5 members of different age groups are able to earn Rs.75000-120000 in a season of the year and this amount is adequate for their livelihood without migrating to plain areas and this also supports the observation of Arif & Kumar, 2003; Das *et al.,* 2005; Negi a, b and c, 2006; Negi *et al.,* 2003; Garbyal *et al.,* 2004 and Sharma, 2004.

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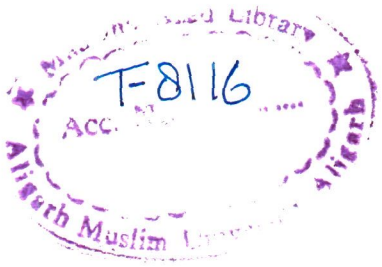

Dedicated

to

my Parents

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DEPARTMENT OF ZOOLOGY
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INDIA

Sections :
1. ENTOMOLOGY
2. FISHERY SCIENCE & AQUACULTURE
3. GENETICS
4. NEMATOLOGY
5. PARASITOLOGY

D. No. ...../ ***ZD*** .....

Dated .....

Certificate

This is to certify that **Mr. Shahid Sami Siddique** has completed his Ph.D work under my supervision on the problem entitled **"Effect on Socio-Economy and Bio-Ecological Relation of Yartsa Gumba – An Entomo-Fungal Complex in Pithoragarh Himalaya".** The work is an original contribution and distinct addition to the existing knowledge on the subject. Being satisfied with quality and quantity of the work, he is permitted to submit it for the award of the degree of Doctor of Philosophy in Zoology of the Aligarh Muslim University, Aligarh, India.

30/11/16

(Prof. Shujauddin)

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Ssami

Shahid Sami Siddique

CONTENTS

Chapter-1

Introduction

INTRODUCTION

Yartsa gumba or dbyar-rtswa-dgun-bu is Tibetan name of an Entomo-Fungal combination between *Hepialus armoricanus* (Oberthur). (Lepidoptera; Hepialidae) larva and its parasitizing fungus *Cordyceps sinensis* (Berk), which is traditionally used in Tibetan and Chinese System of Medicine (TCM). This medicinal entomo-fungal product is known in Chinese as Dong Chong Xia Cao (winter worm and summer plant or grass in summer and worm in winter), Yartsa Goenbub in Bhutan, Yarchagumba means herbs of life in Nepal and Tochukaso in Japan. It is also known as Caterpillar mushroom, Caterpillar fungus, vegetable wasp, plant worm, vegetable caterpillar (Cooke, 1892) and in India it is commonly known as Keera Ghas. This entomo-fungal combination is known to be used for many centuries as tonic, medicine and aphrodisiac and in religious ceremonies in China, Indonesia and Upper Himalayas. The medicinal importance of this combination is due to a fungus viz., *C. sinensis* parasitizing the host caterpillars i.e. *H. armoricanus* (Arif & Kumar, 2003).

In nature host *H. armoricanus* and parasitizing fungus *C. sinensis* both occur in difficult terrain of high altitude areas ranging from 3000-4571m altitude and are rare. Its occurrence in high mountains at an altitude above 4000 m was noticed and reported by Garbyal (2000 & 2001). Exhaustive work has been done on high altitude insects and its biology by various workers (Mani, 1995; Arif, 1995; Arif & Kumar 1995; and Arif & Kumar 2002). Native occurrence of the fungus is confined to high Himalayan mountains in Tibet, Nepal, China, India, Bhutan and Sikkim at an altitude ranging from 3000-5000 m. The most common

occurrence of this fungus is between 3500-4500 m elevation in cold and arid environment (Sharma, 2004)

C. sinensis, a parasitic fungus in the alpine regions, is highly valued in the traditional medicinal system of China, Nepal and India. However its high medicinal value is known in recent past in India. Collection from wild habitat is a new income generation opportunity in the remote locations of the central Himalayas regions. Keeping in view the high cost of wild collection as a raw material, local people along with their entire family move up with their sheep's and goats during May and June and they because of their expertise start watching, spotting and digging out the Yartsa gumba.

Since 1999-2000 it has been under big exploitation as a raw material without knowing any ill consequences on the impact of the environment, forcing insect in endangered stage and its own extinction (Arif & Kumar, 2003). During the digging and collection of the infected stages of the caterpillars, pupae and uninfected stages of *H. armoricanus* get killed knowingly or unknowingly and most of them get exposed as food of birds. Since beginning insect as a whole or its products are used as food, clothing, wax, honey, lack, dyes and medicines (Allotey, *et al.*, 1997; Frost, 1994; Gullan & Corastan, 2000). Besides its beneficial action like pollination, predation and parasitism, insect and fungus relationship is well known in the form of host and parasites on the basis of which commercial exploitation of fungus started as microbial control of insects pests (Metschnikoff, 1879 and Srivastava, 1988).

So far, various workers (Garbyal, 2001; Sharma, 2004; Negi, 2003 a, b, and c; Negi, 2006; Negi, *et al.*, 2006 and Arif & Kumar, 2003) have explored different locations of Dharchula and Munsiyari, however, these workers neither explored Laspa and Ralam dhara of district Pithoragarh

area nor they correlated the socio-economy of the locals related to local collection of Yartsa gumba. Keeping in view the above problem there is urgent need for scientific exploration on the possibilities of rearing of insect, inoculation of specific parasitizing fungus, growing of *C. sinenisis* through biotechnological method for the improvement of socio economic status of the local inhabitants and the conservation of the environments.

There are frequent complaints regarding contamination to the natural specimens with grass sticks or some heavy metals by inserting wood/grass stick, steel wire or nails to increase the weight and moreover, there are ethical issues related to consumption of Yartsa gumba being an insect. Further continuous exploitation of Yartsa gumba in nature as wild harvest will disbalance either of them and ecosystem and environment as well. Moreover, it's rearing in nature and controlled condition is not an easy and possible way. Therefore, the mass rearing production through *in vitro* culture is a suitable and economic option, which has been planned, to carryout in the present study.

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*Cordyceps used in the present text is equivalent to the Yartsa gumba (Insect-Fungus complex). However, *Cordyceps sinensis* (Berk) is referred as fungus species.

Chapter-2

Historical Review

HISTORICAL REVIEW

The first ever report of the Yartsa gumba dates back to the eighteenth century when Torrubia a Franchisian Friar in Cuba described it as the trees growing out of the bellies of wasps. This is why Cordyceps is sometimes known as Torrubia in the honour of its inventor (Christensen, 1975). Cordyceps is one of the most rare and treasured herbs and it has been an important ingredients in Chinese medicine for thousand of year. It is found in isolated places in south western China especially in the provinces of Tibet, Sichuan, Qinghai, Guise and Yannan in locations over 3500 meters altitude. Traditional knowledge to explore the fungus at high altitude of Tibet and China is to watch wild yaks, usually the mushroom is found where the Yak grazes.

During the time of open Border between Indo-Tibet, before Indo-China war in 1962, traders of the Himalayan region in India visited Tibet-frequently. Prevailing traditional knowledge among the traders was the use of this fungus to make their pet animals (carrying loads) energetic during travel in high altitude. Cordyceps known to the Chinese as Dong Chong Xia Cao and to the Japanese as Tochukaso has been used in medicine for a long time. The first time written record of this herbal medicines was in the Ben-Cao-Cong- Xin (New compilation of Materia Medica) by the author Wu Yiluo written around the year 1757 during the Qing Dynasty (Zhu, 1998) and in this early medical text traditionally usage of Cordyceps has been listed as useful as a 'Lung Protectorate' for kidney improvement and as a "Ying/Yang double invigorant" Cordyceps in Traditional Chinese Medicine (TCM) was usually still prepared by cooking the whole caterpillars/fruiting body combination in chicken or duck soup (Tiera, 1998). It has been used this way for the treatment of

many diseases such as respiratory diseases, renal dysfunction, hyperlipidemia and hyperglycemia. (Zhou *et al.*, 1998).

Review of literature reveals that Cordyceps has been reported to cure more than 200 disease like genetic asthama, cancers, antiagings, prosexual, aphrodisiac, immunologic, erythropoetic, antineoplastic, antiarrythymic, hypoglycemic effects etc (Arif and Kumar, 2003). Beside, it is also used as aphrodisiac an important nourishing tonic energy booster and adaptogen, which increases physical stamina by enhancing oxygen, supply to the brain and heart and thus improves the resistance to the hypoxia. This was recommended by ancient practitioners as 'Panacea of all ills', however, it got attention of people when Chinese athletes set the world record in Olympic games in 1993 who were found administered the doses of compounds extracted from Cordyceps. It is reported to have tonic astringent, expectorant and antiasthamatic properties. It is believed to tone up kidney and to be useful for weak back and knees, impotence and other deficiency symptoms. It is also said to be good for lung.

Cordyceps was discovered about 1500 years ago in Tibet by herdsman who observed that their livestock became energetic after eating a certain grass like mushroom even the older animals become vigorous and more youthful in their actions. About 1000 years later emperors physician in the Ming Dynasty learned about this Tibetan wonder and used this knowledge with their own wisdom to develop powerful and potent medicine (Sharma, 2004).

In Tibetan medicine system Cordyceps valued very highly and used to increase vitality and in restoring regenerative fluid, especially the fertility of sperms, kidney and heart. It is also known to suppress r-lung (vata) and alleviate m-khrispa (Pita). Tibetan mixture Cordyceps with alcohol or traditional green tea and drink is for vitality and to cure

stomach ailments and this is considered to be very safe drug and care be taken for extended period of time.

Tibetan scholar wrote detailed description of Cordyceps in 15th and 18th century texts. Cordyceps was introduced to Europe at a scientific meeting in Paris in 1726 and first imported to Japan in 1728 (Sharma, 2004) for the traditional use of Cordyceps to improve circulation as well as health of lungs, heart, kidneys and liver.

The Tibetan name Yartsa gunbu (dbyar rtswa dgun bu) means 'Summer grass winter worm' apparently grass (rtswa) is also found to denote other mushroom such as *Ganoderma lutescens* which is also collected as a medicinal mushroom for the Chinese market in some Tibetan areas. Boesi (2003) noted that this term describes the life stages of Cordyceps. Ancient Chinese, about 2000 years ago, are said to have placed stone effigies of insects with *C. sinensis* in the mouth of their dead hoping to revive them or to prevent decomposition as in the care of fungal mummified insects (Gee, 1918; Holfmann, 1947; Mains, 1958; Kobayashi, 1941; Mc Coy, 1988; Steinhaus, 1956; Tiera, 1998 and Chatterjee, 1957). In China it is also believed that Cordyceps when boiled with pork, cures opium addiction, poisoning, Jaundice and even tuberculosis (Gee, 1918). The historical uses of Cordyceps as an antiaging herb in traditional Chinese Medicine (TCM) dates back to 1700 B.C. during China's Chin Dynasty. One of the emperors is said to have paid an ounce of gold for a three days supply of the precious fungus.

In India particularly in Dharchula and Munsiyari areas of district Pithoragarh local people consumes Cordyceps with alcohol. Cordyceps are immersed in local breavage alcohol for some time before consumption. At the Stuttgart world Championship Chinese women took gold medals for the 1500-3000 and 10000 meter events and set new world

records. Due to use of an amazing medicinal extract of Cordyceps there has been an international sports record. A team of Chinese women runners shattered nine word records, breaking the records for the 10000-meter run by an unprecedented 42 second (Chinese National Games Beijing 1993). The female Chinese long distance runners surprised the world by winning all the distance events at the world outdoor track and field championship in Germany. Their coach (Ma Zunren) attributers their amazing performance to intensive training and a special stress relieving tonic (diet) containing Cordyceps prepared from caterpillar fungus. At present Cordyceps is taken by a number of athletes for endurance. In other examples a Boston Marathan runner who had been taking Cordyceps cut an unbelievable 25 minutes of his time and placed in the top ten winners.

In the beginning, collection and trade of caterpillar fungus from Tibet to China was done for the exchange for tea or luxury goods like silk, confirming its economics importance. Bacot (1912), Wilson (1913) and Bailey (1945) brought the first photo of Yartsa collection taken in Lithang in 1911 to the West. In the spring and summer months Tibetan also dig plants and collect fungi and other articles of medicinal value for export to the Chinese market.

Historically 60% of China's Chong Cao supply came from the Tibetan areas of Sichuan. During the cultural revolution and the commune phase the Chong Cao market cooled off, however, it resumed with the economic liberalizations in the early 1980s and since then caterpillar fungus has developed into one of the most important 'Cash Crop' on south Eastern plateau. Its small size, easy preservation and high value made transportation very easy. Dried specimens offered much higher price in winter. Cordyceps is traded in several categories. The

Chapter-3

Materials and Methods

MATERIALS AND METHODS

3.1. EXPEDITION TO TARGET AREA

Before the expedition to study area, author of the thesis along with 4 helpers were imparted training by the Commandant, Indo Tibet Border Police, Pithoragarh. Training comprised of survival ration, required clothing's, protection from adverse climate, load carriage, tentage fitting, cooking inside tentage, trekking, crossing water streams, climbing rocks and movement through forest, rocks, pebbles, stones and medical combat from physical/mechanical injury or climatic cold stress etc. during 2004, 2005 and 2006. Expeditions were lead by the author during May-June in 2004 and April-May -June-July 2005, and May-June in 2006 to take observation on different stages of *H. armoricanus* host insect and its host plants *C. sinensis*. Expedition were taken to four different locations viz., laspa Brizgang area (13500-14000 feet), Ralam glaciers (14500-15500 feet), Khelach (11000-11500 feet) and Tola at an altitude of 10000-11000 feet (Table 1 and Fig. 1). Local farmers of the remote villages of Munsiyari Block of district Pithoragarh were contacted and their traditional experience of collection of Yartsa gumba was shared after a big halt. Block Pramukh, Gram Pradhan and Forest authorities were also contacted to have their permission for study and shared their experiences

During different expeditions two types of tents i.e. Light weight (Fig. 5) and Heavy weight (Fig. 6) were taken depending on the area of study. Tents were fitted and at night gas petromax was used for light and drying of fresh specimens. Expedition stayed for 6 days in each study area and from the camping place collection was made in an area of 1 km periphery of the tent. Yartsa gumba were spotted out with the help of the naked eyes and dug out with the help of especially designed kudal. Some of the specimens were kept in 70% alcohol and some of them were

wrapped in cotton after cleaning the soil. Their measurement were taken on the spot in fresh conditions to study the different stages of *H. armoricanus*. In all total 15 numbers of pits measuring 1 x 1 x 0.40 m were dug out and different live and dead stages of larvae were collected and counted. The depth of their occurrence was measured. During keen vigil and watch, eggs of *H. armoricanus* (Fig. 37 and 40) were collected. During collection by local farmers, observation was recorded on the mortality of insect stages at the time of digging. Having stayed in 24 villages of Munsiyari blocks, family members were contacted and their livelihood was studied in details. Total population of each village was collected from the office of the Block Pramukh, Munsiyari of district Pithoragarh (Table 9). Source of income of villagers were enquired, observed on ground and recorded. Further interaction was made to know about the enhanced income after Yartsa gumba venture.

3.2 OCCURRENCE & HABITAT IN INDIA

Yartsa gumba is endemic to the Tibetan plateaus adjoining to high altitude areas of the central and East Himalayas (Nepal, Bhutan, Uttaranchal, Himanchal, Arunanchal and Sikkim in India). Its distribution is limited to the area with an average annual rainfall above 350-400 mm.

Cordyceps sinensis occurs in alpine meadows at high altitude areas and recorded its distribution to its host *H. armoricanus* to Sichuan, North Yannan & vast areas of Tibet, Lunana and Gasa, Djongkhaj village and Jumla and Dolpa areas of Nepal, Sichuan Qinghai Guise and Yannan in western China at an altitude of 3500 m, Tian Shan and Altai Shan in Xinjiang North-West China Soe, Lingshi and Laya areas of Bhutan, Upper valleys of Phutey covering Tkanza, Tenchey and Tshodzong villages of Bhutan and Sikkim. In India Yartsa gumba occurs mainly at alpine meadows stretched in 10-15 km areas of Munsiyari and Dharchula

Block of district Pithoragarh of Uttaranchal. It's main habitat is Chiplakot, Brahmkot, Ultapara, Ghwardap. Sumdum, Baling, Dugtu and Dantu in Darma valley, Chhipla, Malpa Top, Nijyang Top Karsrchila, Budh, Galja, Chal, Baling, Bon, Dugtu, Panchachuli, Najari, Martoli, Nampa, Api at Dharchula Himalayas and Laspa Tola Ralam glacier-Brijgang, Cherthe, Ralkot, Khelach. Nagi-Dhura and Cherthy Dhura is most preferred site for fungus gatherers and Khelach at an elevation of 11000-11500 feet altitude in Munsiyari Block (Sharma, 2004; Garbyal, 2000 & 2001 and Negi, 2003)

However, there is report of its occurrence in Nandadevi and Lahual spiti in Himanchal Pradesh. Cordyceps proliferate on well-drained sunny slopes with lush green vegetation sites, which are too wet or waterlogged. The bogs and tussock grass areas do not have Cordyceps population. During survey of the present investigations when the snow starts melting (Fig. 7) and waters the vegetation of the slopes in the presence of sunlight, fruiting bodies start coming up (Fig. 15).

Table: 1 Place of Collection & their altitude in Munsiyari area of District Pithoragarh.

S.No.	Place of Collection	Altitude feet.	Year of collection
1	Laspa (Brizgang)	13500-14500	May-June, 2004
2	Tola	10000-11000	April-May, 2005
3	Ralam Glacier	14500-15500	June- July, 2005
4	Khelach	11000- 11500	May-June, 2006

Chapter-4

Results

RESULTS

The Yartsa gumba (an entomo-fungal combination) collected from different areas located at different altitude viz., Laspa-Brizgang area (13500-14500 feet), Ralam glacier (14500-15500 feet), Tola (10000-11000 feet) and Khelach (11000-11500 feet) are presented in Table 1-5. Detail parameters on Yartsa gumba viz., total length of entomo-fungal combination, length of caterpillar, stalk length, fresh weight of Yartsa gumba with soil, dried weight and drying percentage are given in Table 6-8. Specimens were collected and dried in oven at 56^0C and data in both the conditions were recorded in detail.

Occurrence of larvae and pupae of *H. armoricanus* were recorded at different soil depth at different altitudes. At Ralam glaciers at 14500-15500 feet altitude (Fig. 8, 9, 10 and 14) and at Laspa- Brizgang area at 13500-14500 feet altitude (Fig. 11, 12 and 13), depth of larval position under soil was 20-25 cm whereas at Tola at 10000-11000 feet altitude and at Khelach area at 11000-11500 feet altitude larval position under soil was recorded at 12-18 cm.

Out of 15 pits dug at four different places, in 3-4 pits none of the larval / pupal stages either infected or uninfected was recorded. Pits were made at a distance of 2 meters to 10 meters and it was recorded that distribution pattern of larval stages depend upon availability of their host plants. However, sporadic distribution of larval stages were observed more commonly. Total number of specimens of larvae and pupae varied from altitude to altitude. It was recorded that total 74, 50, 41 and 25 number of specimens were recorded at 14500-15500, 13500-14500, 11000-11500 and 10000-11000 feet altitude respectively. Highest numbers of 74 specimens were recorded at higher elevation and lowest number 25 specimens were recorded at lower altitude, number of infected

larvae varied from 0-3 at higher elevation (13500-15500 feet altitude), however, this number was recorded 0-2 at lower elevation (10000-11500 feet altitude) per pit. At two locations situated at higher altitude viz., Laspa and Ralam, specimens were not observed in 20% pits and at Tola and Khelach (at lower altitude) specimens were not recorded in 26.6% pits. Percentage of infected larvae from 15 pits recorded were 21.7 % at 14500-15500 feet altitude, 26.0% at 13500-14500 feet altitude, 24.0% at 10000-11000 feet altitude and 34.1% at 11000-11500 feet altitude.

Number of specimens of uninfected larvae was recorded from 0-5 at 14500-15500 feet altitude 0-7 at 13500-14500 feet altitude 0-1 at 10000-11000 feet altitude and 0-3 at 11000-11500 feet altitude. Thus percentage of uninfected larvae was 21.7, 36.0, 12.0, and 22.2 at different altitude respectively (Table 2-5). Number of pupae recorded was 0-7, 0-5, 0-3 and 0-5 at 14500-15500, 13500- 14500, 10000-11000 and 11000-11500 feet altitude, respectively. However, maximum number of pupae was recorded 42 at 14500-15500 feet followed by 19 numbers at 13500-14500 feet altitude. Pupal percentage was observed highest 64.0% at 10000-11000 feet altitude followed by 56.6% (Table 3) at 14500-15500 feet altitude (Table 4).

Average number of infected larvae per pit was recorded 1.06 (maximum) at higher elevation, however, minimum number of specimen was observed 0.4 per pit. Average number of infected larvae 1.06 per pit (maximum) was recorded at higher elevation and minimum 0.2 per pit at lower elevation Average number of pupae was recorded maximum 2.7 at higher elevations (Table 4) followed by minimum 1.06 at lower elevations (Table 3).

During the collection of Yartsa gumba, infected caterpillars were recorded to have one and two stalks (Fig. 16, 17, 18 and 19). However,

the percentage of two stalked specimens is very rare. Out of collection in four expeditions three specimens were recorded to have double stalk from both side i.e. head & caudal region (Fig. 20). These specimens were not dug out instead these were lying above the soil. Specimen's, taken out, were measured in fresh conditions along with their stalk. Specimen varied in length from minimum (6.5 cm) to maximum 11.8 cm, however, after drying their length reduced to minimum 5.5 cm to maximum 9.5 cm. During observation 64% specimens were more than 10 cm in length in fresh conditions, however, after drying 36% specimen were recorded 9 cm and above (Table 8). In abnormal condition total length of specimen is 16 cm with 5 cm caterpillar and 11 cm stalk (Fig.21).

Length of the caterpillar varied from 3.0 cm to 5.5 cm in fresh conditions and 3.0 cm to 4.9 cm in dried conditions. Stalk (Stroma) length was recorded minimum 4.8 cm to maximum 6.5 cm in fresh conditions and 2.5 cm to 6.0 cm in dried condition (Table 8 and Fig. 22 and 23). Fresh weight of specimen was recorded minimum 395 mg to maximum 990 mg having single stalk, however, in dried condition it varied minimum 360 mg to 762 mg. Weight of double stalk specimen was recorded from 1000 mg minimum to 1340 mg. Maximum in fresh condition and in dried condition weight recorded 93.3 mg minimum to 1022 mg maximum. In specimen having four stalks weight was recorded 1575-1665 mg in fresh and 1050-1140 mg in dried condition (Table 8).

Fresh specimens collected from Ralam glacier and Laspa area were washed with the help of brush and clean water, specimens were sun dried properly in open and then total length with stroma, length of stroma, length of caterpillar and weight of dried specimens were recorded. Data are presented in Table 8. Total length of specimens from Ralam glacier varied from 5.9 to 9.2 cm and in Laspa area length of specimens with

stroma was recorded 7.3 cm to 9.4 cm. Specimen with double sided stalk (Fig. 20) was measured 9.0 cm. In Ralam glaciers, caterpillar measurement varied from 3.2 to 4.2 cm as compared to 2.9 to 4.4 cm in Laspa area. Length of stroma varied from 2.5 cm to 5.0 cm in Ralam glaciers as compared to 3.8 to 6.4 cm in Laspa area. Weight of dried specimen varied from 260 mg to 609 mg from Ralam glaciers as compared to 482 mg to 1000 mg in Laspa area (Table 8). However, double stalked from both side of the specimen weighed 948 mg.

Table 2. Population of different stages of *H. armoricanus* in Laspa area (13500- 14500) feet during May-June 2004.

Pits No.	Total Number of larvae and pupae	No. of infected larvae	No. of uninfected larvae	No of pupae	Soil depth of occurrence (cm)
1.	-	-	-	-	-
2.	10	3	2	5	25
3.	2	-	2	-	22
4.	6	3	1	2	24
5.	11	-	7	4	23
6.	2	1	-	1	24
7.	1	1	-	-	25
8.	-	-	-	-	-
9.	-	-	-	-	-
10.	7	1	4	2	25
11.	4	2	1	1	25
12.	1	1	-	-	20
13.	1	1	-	-	20
14.	5	-	1	4	25
15.	-	-	-	-	-
Total	50	13	18	19	22
Average		0.86	1.2	1.26	
%		26.0	36.0	38.0	

Table 3. Population of different stages of *H. armoricanus* in Tola area (10000- 11000) feet altitude during April-May 2005.

Pits No.	Total Number of larval and pupae	No. of infected larval	No. of uninfected larval	No of pupae	Soil depth of occurrence (cm)
1.	2	-	-	2	18
2.	-	-	-	-	-
3.	1	1	-	-	18
4.	2	-	-	2	18
5.	4	1	1	2	18
6.	3	-	-	3	16
7.	1	1	-	-	15
8.	-	-	-	-	-
9.	-	-	-	-	-
10.	-	-	-	-	-
11.	3	1	1	1	12
12.	-	-	-	-	-
13.	1	-	-	1	16
14.	6	1	1	4	18
15.	2	1	-	1	17
Total	25	6	3	16	
Average		0.4	0.2	1.06	
%		24.0	12.0	64.0	

Table 4. Population of different stages of *H. armoricanus* in Ralam glaciers (14500-15500) feet altitude during June-July 2005.

Pits No.	Total No. of larvae and pupae	No. of infected larvae	No. of uninfected larvae	No of pupae	Soil depth of occurrence (cm)
1.	9	2	2	5	22
2.	12	1	5	6	20
3.	3	1	-	2	24
4.	6	-	2	4	25
5.	1	-	-	1	24
6.	4	1	-	3	25
7.	12	3	2	7	22
8.	4	1	1	2	21
9.	7	2	2	3	25
10.	-	2	1	6	24
11.		-	-	-	-
12.	-	-	-	-	-
13.	4	1	1	2	24
14.	3	2	-	1	22
15.	-	-	-	-	-
Total	74	16	16	42	
Average		1.06	1.06	2.7	
%		21.7	21.7	56.6	

Table 5. Population of different stages of *H. armoricanus* in Khelach area (11000 - 11500) feet altitude during May-June 2006

Pits No.	Total Number of larvae and pupae	No. of infected larvae	No. of uninfected larvae	No. of pupae	Soil depth of occurrence (cm)
1.	-	-	-	-	-
2.	3	1	1	1	17
3.	7	2	3	2	18
4.	2	1	-	1	18
5.	1	1	-	-	12
6.	1	-	-	1	16
7.	-	-	-	-	-
8.	-	-	-	-	-
9.	-	-	-	-	-
10.	6	2	1	3	14
11.	2	2	-	-	15
12.	3	1	2	-	16
13.	3	1	-	2	16
14.	6	2	1	3	16
15.	7	1	1	5	15
Total	41	14	9	18	
Average		0.93	0.6	1.2	
%		34.1	22.2	43.7	

Table 6. Total length of caterpillar, stalk and weight of fresh specimen collected from Laspa area (washed specimen).

S.No.	Total length(cm)	Length of caterpillar (cm)	Length of stalk (cm)	Weight (mg)
1.	9.4	4.4	5.0	1000
2.	8.3	3.8	4.5	681
3.	8.1	3.8	5.3	809
4.	8.9	3.5	6.4	598
5.	7.9	2.9	5.0	635
6.	8.6	3.4	5.2	482
7.	8.1	2.9	5.2	730
8.	9.4	3.0	3.8	836
9.	7.3	3.5	3.8	617
*10.	9.00	4.0	5.0	948

*Specimen with double side stalk

Table 7. Weight of mummified caterpillar, length of caterpillar and stroma of samples collected from Ralam dhara (Washed & Dried).

S.No.	Total length of Mummified insect with stroma (cm)	Length of caterpillar (cm)	Length of Stroma (cm)	Weight of dried Insect (mg)
1.	8.2	3.7	4.5	523
2.	9.2	4.2	5.0	609
3.	7.8	4.0	3.8	522
4.	8.0	4.0	4.0	354
5.	6.6	3.7	2.9	431
6.	6.2	3.7	2.5	462
7.	6.5	3.5	3.0	332
8.	7.9	3.9	4.0	369
9.	7.9	4.0	3.9	409
10.	7.8	3.8	4.0	432
11.	7.6	4.0	3.6	539
12.	7.5	3.4	4.1	260
13.	7.7	3.9	3.8	411
14.	7.8	4.0	3.8	534
15.	6.1	3.6	2.5	409
16.	7.3	3.8	3.5	380
17.	6.1	3.2	2.9	362
18.	6.9	3.9	3.0	515
19.	6.2	3.2	3.3	326
20.	6.4	3.4	3.0	282
21.	7.2	3.5	4.2	429
22.	8.2	4.2	4.0	390
23.	6.5	3.0	3.5	270
24.	7.8	4.0	3.8	389
25.	5.9	3.4	2.5	364

Table 8. Weight of freshly collected samples with soil and weight after drying and drying percentage.

Sample No.	Total length (cm)	Length of caterpillar (cm)	Stalk length (cm)	Fresh Weight of specimen with soil (mg)	Dried weight of specimen with soil (mg)	Drying %
1.	10.9(8.7)	4.9(4.5)	6.0(4.2)	798	650	81.45
2. **	10.6(8.4)	5.2(4.8)	6.4(3.6)	1132	940	83.04
3.	6.5(5.5)	3.0(3.0)	3.5(2.5)	395	360	91.14
4.	7.8(6.0)	3.8(3.0)	4.0(3.0)	515	480	93.20
5.	9.3(8.5)	4.5(4.0)	4.8(4.5)	804	670	83.33
6. **	11.3(9.0)	5.0(4.0)	6.3(5.0)	1056	955	90.44
7.	9.9(6.5)	4.9(5.6)	5.0(5.0)	840	763	90.83
8.	10.1(7.0)	4.8(4.0)	5.2(3.4)	641	530	82.68
9.	9.8(7.8	4.8(4.0)	5.0(4.8)	850	677	79.65
10. *	11.5(9.2)	5.5(4.5)	6.0(4.7)	1575	1050	66.67
11. *	11.8(9.5)	5.3(4.8)	6.5(4.7)	1665	1140	68.47
12.	10.8(8.8)	5.0(4.2)	5.8(4.6)	980	762	77.76
13. *	11.4(9.0	5.4(4.3)	6.0(4.7)	1250	963	77.04
14.	10.4(8.6)	4.8(3.6)	5.6(5.0)	880	695	78.98
15.**	8.8(7.0)	5.3(4.2)	5.5(5.8)	1130	946	83.71
16.**	11.4(9.1)	5.4(4.6)	6.0(4.5)	1340	1010	75.37
17.**	10.8(8.2)	4.9(3.9)	5.9(4.3)	1060	933	88.01
18.	9.7(8.4	4.7(4.0)	5.0(4.4)	975	663	68.00
19.	9.5(8.4)	4.7(4.2)	4.8(4.2)	985	672	68.22
20.	9.6(8.5)	4.6(4.0)	5.0(4.5)	868	590	68.00
21.**	11.0(9.0	5.4(4.7)	5.6(4.3)	1133	997	88.1
22.**	11.2(9.1)	5.3(4.6)	5.9(4.5)	1250	1022	81.76
23.**	11.2(9.0)	5.2(4.6)	6.0(4.4)	1058	977	92.34
24.	10.2(8.9)	4.4(3.9)	5.8(5.0)	870	697	80.11
25.	10.6(9.0)	5.2(4.9)	5.4(4.1)	990	754	76.16

Data given in parenthesis is after drying of specimens

* Specimens with four stalk (two stalk each side of head and caudal region).

** Specimen with double stalk.

Chapter-5

Discussion

DISCUSSION

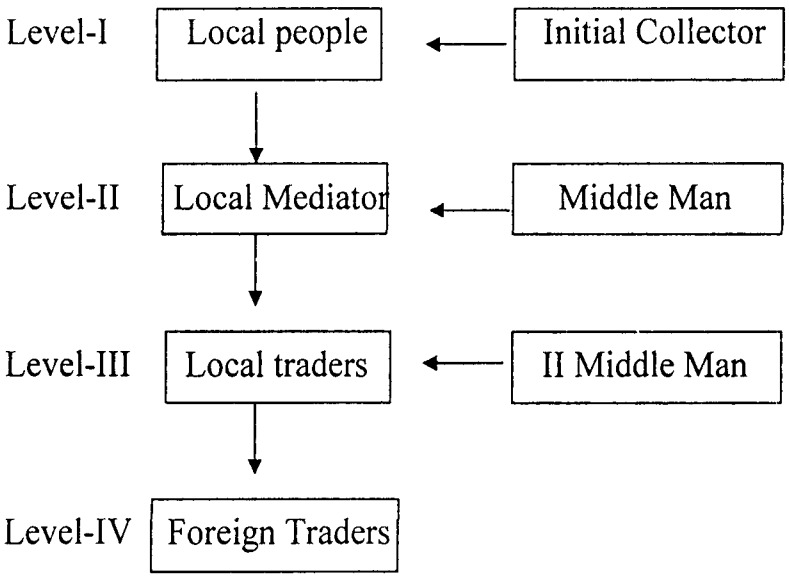
5.1 COLLECTION OF YARTSA GUMBA

Yartsa gumba is primarily collected from wild occurrence as the grown Cordyceps is of lower quality but the wild Cordyceps is usually very expensive and cost upto $1000 for 100 g. Wild Cordyceps from Tibet is supposed to be best in the world. So far there is neither the people are identified nor any standard protocol has been developed. Even local people are not trained to identify infected/uninfected caterpillars, stages of insects and other than host caterpillars. Alpine meadows above 9000 feet are entirely covered with snow from Oct-Nov to mid April every year. Entire flora and fauna remains under the snow and local people come down to their main home located at about 5000-6000 feet. As soon as snow starts melting local farmers with their entire family, pets and sheep's /goats start moving in the month of mid May to reach the target area. There are more than 3-5 stoppages in the form of boundarised area made by stones, wood in open and covered by polythene where they reside inside. Most of the stores are transported by sheep's and goats for their use to sustain for 2-3 months.

In India this fungus was popularized a decade back when it was collected by some local people called Khambas (a Tibetans race) in high altitude areas of Dharchula (Pithoragarh) of Kumaon hills in Central Himalaya. Keeping in view its medicinal value in mind, a large number of local people from nearby areas started visiting at such an inaccessible area for collection regularly. Since there is no demarcation for collection village wise, so to avoid dispute, areas are defined by their own village panchayat. In recent past eligible passes/permits have been issued to Schedule Tribes and Schedule Caste community by the forest authorities.

For collection stalk of Cordyceps requires very sharp eye to locate it among its host grasses. In summer the stalk gives it the appearance of grass (Fig. 15) and hence the name dbyar-rtswa-dgun-bu which literally means grass in summer and worm in winter. Yartsa gumba is extracted during May- June before the onset of monsoon. Because of antibiotic properties of cordycepin the larva does not decay and remain intact. Gatherers have kudal, steel wire and long screws devices to dig out the Yartsa gumba. They first sit down on the ground (Fig. 26 and 27) and some time lie down on the ground to locate the stalk and take out the Yartsa gumba and keep all collections in shallow aluminum plates so as to get the collection exposed and dried in nature (Fig. 29 and 30).

Fresh specimens are covered with soil and caterpillar is some what loose (Fig. 31) and swollen. After collection, specimens are kept in open and dried in nature. These specimens are directly sold to local mediator among them at the cost of Rs. 80000/ kg on the spot in alpine meadows and local mediator transports it to roadside where there collections are sold to Indian traders of Dharchula and Munsiyari @ Rs 100000/kg. Finally collections are sold to Tibetan traders of nearby market, Taklakot @ Rs, 1,20000-1,50000/kg depending on the production, collection and availability. Thus there are three stages of collection and disposal in India connected by 4^{th} stages of foreign traders a given below:



Local people are given authorized pass by District Forest officers with necessary instructions. Collection is done under the vigil and watch of forest guard and local patwari so as to have an estimate of collection and to follow the instruction and to avoid local disputes. Usually I-III level gatherers are open, however, IV level gatherer are secret so as to avoid taxes and international legal problems related to trader. Only a few traders are authorized by the Indian government but during the season there are a number of unauthorized traders, present in the area of collection and main market. Depending on the size of specimen there weight varies from 260 mg to 1160 mg and one kilogram contains 2000-5000 specimens since specimens are of mixed size and weight so there number varies. However, graded size of higher weight may contain 1000 specimens per kg.

According to Sharma, (2004) Primary gatherers (mostly villagers of the valley) stay in alpine regions for several days and collect the fungus. During the growing periods of the fungus the main brokers from the regional market send their employees to various localities for collection of material. Other than this channel, various mechanism have

been developed. Few more independent agents also work, but the final destination for most of he collected material in that area is usually a regional brokers. Gathering information about the collection, route and market is difficult because it is secret type of mission.

A new trend has been observed in the inner mountain ranges of the central Himalayas, where collection and selling ranges of the Cordyceps has emerged as a new source of income in the rural areas. In the river valleys of Gori Ganga alone, the number of fungus gatherers at alpine habitats has increased about four fold since the year 2000. Total 900 persons visited in seven different habitats in 2000, thus 128 person per habitat visited with total collection of 186 kg. This came to 200g/ gatherer costing Rs.8600/gatherer. Price increases to Rs.7000 after carrying material few kilometers down to the local market. Purchase price has also increased at the field site and in the local market tremendously and so the income of gatherers of wild materials between 2000-2002 increased by 3.7 fold to 4.0 fold. Thus selling price was 68000-80000 in Tibet, Rs 80000-90000/kg in 2000 in Nepal and Rs 1.25-1.30 lakh /kg in India.

5.2. GENUS *HEPIALUS* (SWIFT OR GHOST MOTH)

Hepialus belongs to the family Hepialidae of Lepidoptera containing 500 species from the world. Adults are small to large in size, diurnal, crepuscular or nocturnal moths whose proboscis is short or absent. The family is cosmopolitan, though about one quarter of the species occurs only in Australia. Larvae are subterranean and live in vertical tunnels excavated either in wood, feeding on re-grown bark at the entrance, or in soil where they feed on roots of grasses or emerge to eat low growing foliage or leaf litter and some are important grassland pests (Ross, 1965; Grillot, 1980; Metcalfe & Flint, 1990 and Arif & Kumar,

2003). The hepialid in New Zealand are well known by the common name Porina which strictly refers only to the pasture pest genus Weiseana. The economic damage to pasture grasses caused by the feeding activity of Porina caterpillar is second to that of grass grubs. There are few species of *Hepialus* viz., *Hepialus hectus* (L.) (Gold swift moth), *Hepialus humuli* (L.) (Ghost moth), *Hepialus sylvania* (L.) (Orange swift moth), *Hepialus lupulinus* (L.) (Common swift moth) and *Hepialus flusconebulosa* (Be Geer) (Jamieson *et al.*, 1999). *H. lupulinus* completes its life cycle in 2 years.

The normal reproductive cycle for *Thitarodes* (=*Hepialus*) takes upto 5 years out of which most of the life cycle lived as caterpillar, the moth itself live for only a short time, 2-5 days in the case of *Hepialus biruensis*. (Chen *et al.*, 2002). As many other ghost moths, the adult of Thitarodes moth is not able to eat. The host for *C. sinensis* most commonly reported is *Thitarodes armoricanus* (Oberthur) under family Hepialidae of Lepidoptera. Other host larvae have been identified such as *Hepialus oblifurcus*, (Chu). *Hepialus baimaensis* (Liang) and *Hepialus biruensis* (Fu-Huang) (Chen *et al.*, 2002). Nearly 40 species of *Thitarodes* (*Hepialus*) moths are recognized in the 'Tibetan Plateau' region. According to Chen *et al.* (2002) 30 species can be infected by *C. sinensis*. Chinese entomologists are still using the generic name *Hepialus*, although it has been restricted to a single European species *H. humuli* for some thirty years. The genus *Thitarodes* was erected in 1968 to accommodate *H. armoricanus* and other related species placed originally in *Hepialus* (Nielsen *et al.*, 2000), many of them are the host for *C. sinensis*.

5.3. CATERPILLAR (LARVAL STAGE)

Young larva hatches out from egg in the grass litter and starts their development on the host plants. Grehan and Rawlinsa (2002) reported that larva forms a typical '7' shaped tunnel and a silk covered external feeding scar over the entrance. The larva moult into three phases in which they complete their growth. The entire larval period may take as much as 4 years and mature larva may exceed 100 mm in length (Fig. 17)

Larva digs a burrow more or less vertically into the soil. The soft phase larva lines the burrow with silk and construct a silken runway from the entrance out to its feeding area. Larva emerges at night and cut grass shoot at the base or aerial parts of the plants and drags them back into the burrows so as to eat later. The life cycle is completed in one year. In Tibetan medical literature it is described as one with slender, short and bamboo shaped root which has smoke or ochre colour with light yellowish or skin colour exterior and white fleshy interior. The root has worm like head, body and legs with numerous thin and fine transverse wrinkles. There are about eight pairs of legs on the body of the root and out of them four middle pairs are more prominent (Fig. 18). Larval stages viz., 2^{nd}, 3^{rd}, 4^{th} and 5^{th} instars and eggs collected were studied (Fig. 33, 34, 35, 36, 37, 38, 39 and 40).

The host plants of caterpillar in alpine meadows are grasses and plants including, dock, nettle, dandelion and burdock (the local herbs of high altitude habitat) roots of several lower plants including *Plantago*, *Urtica*, *Rumex*, *Solidago* and *Fragaria* are also eaten by the caterpillar.

Their lower part is thin while upper part is slightly thicker. Caterpillars collected from different location were observed under Zoom Binocular Microscope where 3 pairs of thoracic legs, 4 pairs of

abdominal legs and caudal segment are modified into caudal legs (Fig. 17 and 18). Thus caterpillar of *H. armoricanus* posses 12 segments with thoracic and abdominal legs more prominent as compared to other lepidopteron caterpillars. Present observations support the findings of Arif & Kumar (2003).

Infected and uninfected caterpillars are often observed in soft soil under trees in mountains over 3500-5000 m altitude or in cold and well-drained grassy marshy land. The life cycle of uninfected caterpillars take one years to complete. In present investigation it was observed that freshly harvested caterpillars are encased in a black topsoil layer (Fig. 31 and 32). During collection, soil coating is first opened by hand or commonly with a toothbrush, which exposes the caterpillar's body. Gatherers are very careful while digging out the specimen and their cleaning to avoid breaking of stroma, which reduces the cost of Yartsa gumba.

5.4 PUPA

Pupation takes place inside the soil when the temperature goes down and snow fall start. All larval stages further goes deeper under the soil for pupation. Final instar caterpillar makes burrow lined with silk and prepare an earthen cell where in caterpillar pupates (Fig. 41, 42, 43 and 44). Remaining stages remains inside the burrows lined with silk or remain hidden under tunnel or excavation of the roots to avoid climatic stress.

5.5 MOTH (ADULT)

Imms (1973) reported that moths of all hepialids are difficult to obtain in perfect condition. In adult, antennae are very short and mouthparts are vestigial. *H. armoricanus* are mostly moderate size with

average wing span of 45-70 mm. However the size may vary from 35 mm to 150 mm. Wings are usually large and strong and many species are known to be swift fliers. However, these do not tend to disperse far from their place of origin, life span of the adult is brief.

Most of the *Hepialus* adults emerge in spring or early summer. Adults may be on the wing between September and April, depending on species and altitudinal locations. Usually adults emerge and fly in the late afternoon or around dusk and may be on wing for only an hour or so, especially the males. Most species emerge in warm and misty weather. Male differs markedly from female in colour. In *H. humuli* the males being commonly white and is readily sought out by the females. (Ealand, 1984 and Imms, 1973).

5.6 GENUS-*CORDYCEPS*

The genus *Cordyceps* is classified as under:

Subphylum - Ascomycotonia

Class - Pyrenomycetes

Order - Clavicipitales

Family - Clavicipitaceae

Genus - *Cordyceps*

New classification of *Cordyceps* spp. has been suggested on the basis of chemotaxonomy of partial nucleotide sequences of 18S rDNA obtained from four different species (Ito & Hirano, 1997). Among various species of genus, *C. sinensis* is highly valued in the traditional medicinal system of China.

Kobayashi (1941) listed 137 valid species under the genus *Cordyceps* and of these 125 were recorded as parasitic on insects order

viz., Lepidoptera, Diptera, Hymenoptera, Coleoptera, Hemiptera, Isoptera, Orthoptera and Spiders. Dube (1983) has recorded 200 species of genus *Cordyceps* which are known to be parasite of insects. The genus *Cordyceps* is the oldest genus of the family Clavicipitaceae recorded in literature as plant worm, vegetative wasps, trees growing of insect. *Cordyceps militaris* (Linn) have been unsuccessfully used as insect biocontrol agents by Von Tubeuf (Mc Ewen, 1963). Further, dried fungus has been grown on the larvae of *H. armoricanus* (Oberthur) (Huang, 1999). Some of the well known species of *Cordyceps* are *C. multiaxialis* (Mu Zang), *C. nepalensis* (Mu Zang), *C. Canadensis* (Linn), *C. memorabilis* (Cesati), *C. soboliferea* (Berk), *C. cicadae* (Berk), *C. forquignon* (Quelet), *C. capitata* (Link), *C. militaris* (Linn), *C. ophioglossides* (Link), and *C. sinensis* (Berk) (Sarbboy, 1996; Jagadde & Patil, 1983 and Kinjo & Mu Zang, 2001).

Normal range of occurrence of this *C. sinensis* is at 2000 m. elevation, however, it has been observed as high as 6000 m altitude. It is found for a short time each summer, growing on its natural host.

Sung (2004) reported 300-400 species of *Cordyceps* of which 68 species have been recorded in China (Wang, 1999) and 33 species in Tibetan Plateau and Himalayan region (Zang & Kinjo, 1998). *Cordyceps* spp. parasitize larval, pupal and adult stages of insect of the order Lepidoptera, Hymenoptera, Diptera, Orthoptera, Coleoptera and Spiders and sometimes deers. However, only a few are collected for their medicinal properties and most commonly used among these is *C. sinensis*.

5.7 PARASITIZATION OF CATERPILLAR OF *HEPIALUS* BY FUNGUS *CORDYCEPS*

C. sinensis grows by infecting larvae or mature insects with spores that germinate often before the cocoon is formed and the fruiting bodies (stromata) germinates from the dead host (Fig. 16,17 and 18). The infection is estimated by the filiform ascospore or their fragment landing on the proper insect host. These spore occur in abundance in the nature and adhere to the integument of the caterpillar while escaping from the adverse climate and going inside the soil for pupation. The fragments become globular and put out a germ tube which penetrates the integument and gives rise to hyphae. The fungus produces chitinase enzyme in vitro (Hubner, 1958). The hyphae break up into fragments that get distributed in haemocoel and the fragments bud off more propagules which give rise to hyphae. Soon the insect gets stuffed with the mycelium and consequently the host become sluggish and die. The mycelium modifies and develops into a scleroteum which remain covered by the integument of the dead insect. The cadaver of the insect enlarges in size and becomes resistant to decay due to toxin-antibiotics (cordycepin) produced by the fungus (Dube, 1983; Nair and Balakrishana, 1995; Zhou *et al.*, 1998; Hobbs 1986 and Lie *et al.*, 1999). Life cycle of *H. armoricanus* is given in Fig. 45.

The antibiotic (cordycepin) released by parasitizing fungus keeps insect free from bacteria and thus infected caterpillar does not decay. This antibiotic is also used in molecular biology to block RNA synthesis. The cadaver with endo sclerotia lie buried underground until the favourable spring weather condition for sclerotial germination are available and thus the fungus survives the winter in this stage usually in or on the surface of the soil (Fig.45). In the spring club shaped orange coloured stroma with a

stalk and head arise above the ground and bear peritheca in the periphery of the head region. In section it was observed that the asci have a characteristic apical apparatus containing 8 filiform ascospore (Fig. 51 and 52), which are released violently in succession. The ascospore multiplies by division into small segments and get dispersed by wind.

C. sinensis infects to host through integument usually when the epicuticular wax is for one reason or another removed or abraded. Symptoms of a fungal disease infection include behavioural symptoms such as ascending plants stems and foliage which seem well suited to efficient dispersal of the fungal spores. Restlessness is a common symptom for parasitized larvae and thus causes them to wonder away before climbing and dying. Paralysis is also a very common symptom, along with colour changes to creamy black or brown in the infected larvae. When the dying larvae climbs up a grass stem it dies attached to its support by its paralyzed legs or tied by the fungus hyphae growing from its body. (Jolivet, 1998). Continued hyphal growth within the body produces a kind of *pseudosclerotium* and bears peritheca and conidia (Carlile *et al.*, 2001).

In the present study it was observed that the larva gets infected by the fungus at the end of autumn and therefore in winter it is still alive and gradually infects the entire body and covers the whole larvae and subsequently kills it. It remains buried under the soil up to 14-20 cm deep. As soon as the snow begins to melt in May, the soil softens and stalk appears above the ground (Fig. 45). Stalk interestingly emerges from the dorsal side of the head of the larva (fig. 17 and 18) which resembles grass sprouting or grass blade like structure, however, it differs in colour which is dark blue or black. Usually a stalk is single and from the same region has been recorded. In the present study two stalks from

the same region have been recorded and unusually four stalks 2 stalk each form head and caudal region have also been observed (Fig. 46, 47 and 48) which seems that some caterpillars after infection could not go inside the soil. Stromata are usually reported to be 5 cm, however, in the present study the size varied from 2.5 to 6.4 cm. Dark brown grassy stalk (Stromata) is thickened at the middle with slightly pointed tip and slender base (Fig. 50).

In formerly old days, Chinese people thought that the caterpillar fungus (Dong Chong Xig cao) was worms, however, after years of study it was found that it is really a fruiting body produced by the fungus *C. sinensis* on dead caterpillar, from where the spores are spread in the wind to the next generation of caterpillar. The spores are colourless and grows on caterpillars and pupae burried in the soil in meadows. *C. sinensis* (Berk) parasitize a range of grass root-boring caterpillar which would hatch as whitish ghost moths, when not attacked by *C. sinensis.*

Kendrick (1992) reported that *C. sinensis* have developed a special adaptation to improve their chances of reproductive success. Since reproduction is dependent on a very specific host and each spore fragments into 100 or more part of spores so that each fungal fructification produces 32 million propagules, thus increasing the odds of landing on a larva. Lie *et al.*, (1998) reported that *C. sinensis* produces 30-60 propagules and that usually attach themselves to the larval stage of the insect but also can attach to mature moths. Apparently the larvae are forced by the fungus to move into its final position before being immobilized since the fungus needs proximity to the surface to grow its fruiting body (Stroma) above ground.

The mycelium develops inside the body of insect, first feed on less vital parts, until it has taken over the complete organism filling the

caterpillar with its hyphae. After the insect is completely mummified and emptied of nutrients leaving behind the larval exoskeleton filled with the *C. sinensis* mycelium, the fungus will develop a fruiting body out of the head above the eyes, where the larva has a horn like protuberance in early spring. The 5-10 cm long brown club shaped fruiting body grows above ground to have its propagules dispersed by the wind in order to find a new host insect. The stroma is nearly twice as long as the caterpillar (Fig. 17) when it is fresh and it takes several weeks for the spores to mature. It is reported that fungi usually weigh 75-85% of their weight when dried. In present observation drying percentage is 68 - 93.20% (Table 8).

Since *C. sinensis* grows at the expense of insect internal organs so the size of stroma depends on the size and stage (instars) of the host caterpillar. In the present study the size of host caterpillars varied 2.9 - 4.4 cm in specimens of Laspa area, 3.2 - 4.2 cm in Ralam Dhara glaciers (Fig. 46,47,48, 49 and 50) and size of stromata 3.8- 6.4 cm in the specimens of Laspa area (Fig. 47,49 and 50) and 2.5-5.0 cm in Ralam Dhara glaciers area. Boesi (2003) reported that too much snow during harvest season cause the rotting of stromata and accordingly harvest is lost. In present observation during third collection in the month of June-July because of heavy snow during harvest season delayed the expedition of local collection and even they were forced to remove snow with the help of Belcha to provide suitable climate to grow the stroma. Gatherers started digging to root out infected caterpillars and killing uninfected host caterpillar with low production .The killing of uninfected caterpillar are may have catastrophic in population of the host. There may be the reason of varying size of stromata that due to unfavorable climatic condition, it could not grow properly in higher elevations.

5.8 CHEMICAL PROPERTIES OF YARTSA GUMBA

Yartsa gumba in real is an entomo-fungal combination in which fungus viz., *C. sinensis* parasitize lepidopteran insect viz., *H. armoricanus* both occurring in the same location of high altitude alpine meadows ranging from 3500-5000 m altitude. *C. sinensis* after parasitizing its host caterpillar grow at the expense of entire internal organs of the insect thus we can say it that fungus inside the capsule of insect integument. In this combination both are integral in nature and *C. sinensis* possess medicinal properties.

Cordycepic acid has been isolated from Cordyceps which is an isomer of quimic acid (Chatterjee *et al.*, 1957 and Cannigham, 1951) found in cincona bark from which quinine is obtained. Chaterjee (1957) studied the chemical constituents of Cordyceps and crystalline substance cordycepic acid was isolated (Sprecher, 1963). Chinese studies appeared predominantly on constituents of Cordyceps but mostly the presence of known substances i.e. amino acids, adenosine and palmitic acid (Xu, 1988). Chemically 25-30% of the herb consists of Crude protein, Cordycepin-3'-deoxyadenosine ($C_{10}H_{13}O_3N_6$) and de-mannitol. Cordyceps naturally contains many ingredients such as Adenosine, Uracil, Uridine Guanine, Guanosine, Cordycepic acid, 2' and 3'-deoxyadenosine, Crude protein, Peptide, Vitamin, B1, B2, B 12, E and K, Polysaccharides, Cyclic peptides, Inorganic elements (P , Mg, Fe, and Ca), Peptide, nonhormonal sterols, trace elements, Flavones, 2'-deoxyadenosine, Galactomannas, Polyamines (Spermine, Spermidine, Homospermidine, Putrescine, 1,3-diaminopropane), Glutamic acid, L-tryptophan, L-arginine, Lysine, D-mannitol, Ergosterol, Alkaloids, Fatty acids (Oleic acid, Linoleic, Palmitic and Stearic acids),and Sterols.

There was a greater bio-diversity noted in compounds of different strains of single species of Cordyceps than in almost any other organism analyzed. King Wah *et al.*, (2004) reported nucleoside as the main class of active compounds in Cordyceps and active principles of Cordyceps are believed to come from nucleosides including uracil, hypoxanthine, uridine, inosine, guanosine, adenine, adenosine, cordycepin.

There are a number of other deoxynucleosides produced by Cordyceps, such as compound 2 and 3- deoxyadenosine which is marketed in the United States as a drug for the treatment of AIDS under the trade name of 'Didanosine.' As for as quality of Cordyceps is concerned so for there have been no universally recognized test methods for analyzing this particular supplement. Moreover each company producing / supplying Cordyceps has used different tests, or tested for different substances in order to show their product standing out above all the rest. Analysis is focussed for adenosine, cordycepin, cordycepic acid and for particular sugars or polysaccharides by different workers.Nearly all of the samples of wild Cordyceps analyzed are very similar in chemical composition, but there was a tremendous variation in the secondary metabolite compounds present in cultivated Cordyceps and other species of *C. sinensis.*

Literature reveals the determination of nucleoside, and specifically the deoxy-nucleosides which is most reliable indicators of potency. This class of compounds showed more variation in different samples of cultivated Cordyceps. Many of the deoxy-nucleosides are not found in other organism or at best a very limited number. This compound N6 - (2 hydroxyethyl) – Adenosine is chosen as indicator compounds because it was found in all specimens of Cordyceps and have not found in any other organism. This compound along with Adenosine and 3- deoxyadenosine

(cordycepin) were used in summation as the quality indicator to compare different strains and production methods of Cordyceps. The quantities of the three compounds were added to gather to came up with a numerical quality index for Cordyceps .

5.9 PRODUCTS OF YARTSA GUMBA

Due to its high medicinal properties, products are available in the market of the Western countries as over the counter medicine, tonic, however, the primary source is Tibet. Its products are formulated in the form of tablets, capsules, teas in the name of Cordymax (S-4), CSF-30, CS-81002, CSB-414, Trimyco Gen (TRI), Trimyco Gen Powder (TRP), Trimyco Gen TM, a daily herbal nutritional supplement, MP-7 and Mycoplex-77M. Its uses are suggested as 2-4 capsules of 600 mg/day and thirty drops liquid 2-3 times/day between meals. Cost of the products in European market is $ 7.99 for 75 g, Cordyceps powder,Optygen 90 capsules ($49.95) and Steel Erex 60 capsules ($22.99). However, in India final products of Cordyceps have so far not been developed and usually it is cooked with the vegetables/meat or immersed in the breavage/alcohol. Villagers of Munsiyari and Dharchula are seen to eat one or two yartsa gumba in wild dried form while going for expedition and patients suffering from weakness, cold and cough are given raw yartsa gumba.

There is large number of market worldwide for Cordyceps as a medicine and as a health supplement at high price. Nevertheless there is no standard of quality and well standard cultivation protocols which can be used to produce high quality Cordyceps (Zhu *et al.,* 1998). Marketed products in China is Cordyceps pill (Guangzhou), superior quality Dong Chong Xia Cao (Sichuan), China Cordyceps King, in Korea Dong Chunghacho Green Tea, Dongchunghacho Tea, Powdered Dongchunghacho and Dongchunghacho (cold and dry) and in Japan *C.*

militaris Tablets, *C. militaris* (dry), Lung Nourishing Oral Solution and Energetic Oral Solution.

The strain viz., CS-4 was one of the first commercial strains of Cordyceps isolated in 1982 at the Institute of Materia Medica, Chinese Academy of Medical Sciences known by the Latin name of Paecilomyces hepiali Chen, the aseptically fermented mycelium of their strain under vast extensive human testing and clinical trials during the 1980's and resulted in a commercial product with wide usage in China, known as Jin Shui Bao capsules. More than 200 patients were involved in the clinical trials with CS-4 and the chemical composition, therapeutic activity and toxicity are very well known for this strain (Bao *et al.*, 1998 and Li *et al.*, 2001). A number of other strains have been isolated from wild Cordyceps since then. In traditional medicinal practices, wild harvested plants are considered to have higher, therapeutic benefits thus they have higher prices. Cordyceps has been highly prized for its medicinal properties for centuries and the same tradition still continues. Traditionally, the fungus is traded in China for its weight in silver or gold.

Coales. (1919) described about the trade of the fungus in China for example in 1994 one kg. of fungus was sold at U.S. $ 700 (Steinkraus\ and Whitefield, 1994). So far market price, trade and channels of Cordyceps collection are not transparent in the Indian sub continent, however, commercial trading does exist. In local areas of fungus availability, the price may vary between NR (Nepali Rupees) 30,000 and 60,000 for a Kg. in Nepal. In the market of Tibet and India the cost per Kg. varies and is above Rupees one Lakh /Kg. It is believed that in the international market the fungus may fetch a price between one and two million rupees per Kg. The cost also varies among the trade channels which start from wild material gatherers in the field, brokers and

traders.In Bhutan each Stroma (one plant) weighs about 0.3 to 0.5 g. One kilogram of Yartsa Goenbub is believed to fetch more than Nu 30,000 when sold across the border. Prices varied from $1,000 to $10,000 per Kg. During surveys in the present study it has been recorded that Cordyceps has been traded very extensively in Dharchula and Munsiyari area since 1996, however, it became more commonly known during 1999-2000.The great demand worldwide for Cordyceps and the enormous cost of the wild collected variety has led to many unscrupulous manufacturers and distributors providing adulterated and counterfeit Cordyceps in the world market (Hsu, *et al.*, 2002).

In present studies it was observed that most of the consumers of wild Cordyceps already know that it is normal practice for collectors to insert small segments of twigs or even pieces of wire into the body of the caterpillars to increase the weight. Many consumers of capsulated, Cordyceps do not know what is real Cordyceps even tastes or smells. After testing of some specimens of 'Cordyceps capsules' which contained nothing but rice flour and other samples which contained nothing but flour and nutmeg. Deceptive production practice will be stopped only after standard quality analytical procedures, which is still required as per present observation.

Most of the western world prefers their medicine to come in clean white bottles and need little capsules, rather than in the whole caterpillar form. This makes it even easier and more tempting for some suppliers to sell just about anything under the label of Cordyceps. To identify real Cordyceps, analysis of available Cordyceps both as commercial products and bulk raw material products, grown by nearly all of the cultivators and suppliers worldwide was done and the results were shocking. Almost all of the commercially available Cordyceps product available in the United

States that were imported from China, contained no detectable amounts of Cordyceps and the results of American Cordyceps products were little better. The American grown Cordyceps products consisted almost entirely of unconverted grain substrate upon which the Cordyceps is grown (Wu *et al.*, 1996).

Chapter-6

Ecology, Conservation and Socio-Economy

ECOLOGY, CONSERVATION AND SOCIO-ECONOMY

6.1 AWARENESS AMONG LOCAL PEOPLE

Cordyceps is a fungal infection on the larva and as such it cannot get extinct because of extraction. Fungal spores are always present in the air and soil and when moisture and temperature are suitable they infect the larva. Endanger of *C. sinensis* means entire destruction of vegetation habitat by the caterpillar. As per study (Negi, 2003 a, b, and c; Negi, 2006; Negi *et al.,* 2005 and Garbayal *et al.,* 2004) villagers have noticed that more you collect better the harvest in next year. Collection of Cordyceps does not endanger the environment or the ecology nor it will result in the extinction of any species.

At present wild material gatherers collect fungus from natural wild resources without considering sustainability and use destructive methods. This process leads to poor or no resource availability in future, because of their main aim of maximum collection and harvest for more income generation in unsystematic way. Endemic nature of the fungus along with mass collection attribute from the wild, puts it in the category of threatened plants (Sharma, 2004). In 1992 Nepal banned the collection, use, sale, distribution, transportation and export of Cordyceps.

However, in India there is no regulation and legislation to control over exploitation on the check posts at the forest department hence collection continues, due to its high price and endemic in nature. Moreover, poor people of border have been identified and given licenses in the form permit to collect the wild Cordyceps. Thus, there is big collection, transport and export of material from Dharchula and Munsiyari region of the district Pithoragarh. According to Sharma (2004)

alpine habitats and neighboring ecosystem can be protected to support conservation approach. Under the Protected Areas Network (PAN) of India, following alpine zones have been brought under Biosphere Reserve, National Parks and Wild Life Sanctuaries of high altitude regions of the Himalayas.

1. Nanda Devi Biosphere Reserve in Uttaranchal.
2. Kanchandzanga Biosphere Reserve in Sikkim.
3. Dehan Debang Biosphere reserve in Arunachal Pradesh.
4. Gangotri National Park in Uttaranchal.
5. Govind National Park in Uttaranchal.
6. Great Himalayan National park in Himanchal.
7. Pin Valley National park in Himanchal.
8. Askot Wild Life Sanctuaries in Uttaranchal.
9. Kedarnath Wild Life Sanctuaries in Uttaranchal.

To protect these reserves, Parks and Sanctuaries it is very difficult to watch and ward because of difficult terrain, climatic conditions and limited resources available with the forest authorities. Moreover difficult terrains are usually located at 50-100 km. from the base and there are few permanent guard posts which are to watch and ward for Cordyceps and high altitude animals as well. Occurrence of Cordyceps is a seasonal phenomenon and it is a rare avenue source of income generation in remote areas of the mountains where livelihood options rarely exist and are minimal.

Keeping in view the contribution to the economy of the villagers and use of fungus by traditional medicinal healers, in the recent past, Nepal Government has lifted the ban from its collection which has been welcomed by people living in the backward regions of the country. In India due to lack of awareness on the cost of the Cordyceps in

commercial trade to the villagers, real benefits are not given to primary gatherers who put intensive labour by keeping away themselves in isolation and harsh climate from their family and society on their own, keeping in view the conservation and its sustainability after surveys, the present author recommended two proposals:

(a) Villagers may be educated about proper use of resources and the price be fixed for the material collected by them. There should be a proper training on the identification of different stages of the host caterpillars of the fungus, its habitat, host plants etc. and other insects occurring in the same habitat so as to avoid damage to any stage (egg, uninfected larval stages and pupae) of the host insects and other kind of insects. People should be well trained that in case of digging and if some uninfected larvae/pupae are exposed then those may be properly kept under the soil at required depth. Out of two main regions viz., Dharcula and Munsiyari, people of different villages of Munsiyari Block are regularly trained by the Block Pramukh, invited Scientists, Experts and Forest authorities for collection technique so as to avoid any ill effects on flora, fauna and ecosystem itself as per the observation made in present study.(Fig. 55 and 56).

(b) Grazing by the local herds should be avoided and banned limited for in its occurrence places for the period of digging, so as to avoid damage to Cordyceps its host and plants etc.

6.2. CONSERVATION APPROACH

During wild collection by the local people, knowingly and unknowingly a number of uninfected larval/pupal stages of the host caterpillars are exposed and killed. Thus to avoid this problem biotechnological approach for mass production is a suitable, economic and eco friendly technique. Lyonpo Kenzang Dorji (Bhutan Agriculture Minister), discussed with Kuensel that harvesting will be done in a

sustainable manner and collection should be stopped if sustainable harvesting is not feasible. The Yartsa Goenbub falls under schedule I of the endangered and protected species and should be protected from extinction. According to agriculture Minister Lunana is the only pilot project and lifting of restriction would be a boon to the lunaps and thus it will improve livelihood of about 80 jungs (Households) in Lunana who do not have any other means of income. Committee was formed to monitor the collection and growing areas, price, presence of outsiders and equality for all lunaps. People from lower valleys where Cordyceps hardly grows were also given equal opportunity to collect. A protocol is being developed between Gasa-dzongkhag and the Agriculture Ministry on the harvesting, transporting, marketing and training on sustainable harvesting of the Cordyceps in the jeog. According to protocol only bonafide residents of lunana jeog will be eligible to harvest the Cordyceps and geog Tshogpas will be responsible for monitoring the presence of collectors from other jeogs and from across the northern border. The protocol also says that at least two of every five Cordyceps should be left undisturbed as seed plants for regeneration.

As per the protocol lunaps will sell their collection to the National Institute of Traditional Medicine (NITM), which has been identified as the authorized buyer. The park manager or warden of the Jigme Dorji National Park (JDNP) will seal the Cordyceps and put clear and proper markings on the packets. Thus legalizing its collection would encourage the lunaps to participate in sustainable harvesting and protection of the rare resource. To ensure its protection and sustainability, it is imperative than the local people be given the responsibility as well as benefit of this scarce resource online with the government's policy of community based management. According to the forest and nature conservation rule 2000, illegal collection of Cordyceps can result in a fine up to Nu 50000 or three years imprisonment or both. In present study since the total

population of the remotely located village in the habitat of Yartsa gumba is less thus besides planning for forest rules, local people permitted be kept under strict watch and they should be well trained on the identification of insects stages, host plants and infected specimens to be collected so that entire ecosystem be undamaged. And the dug area be restored during the 10 months gap in off season. Moreover their route of expedition and area of collection will be fixed year to year so as to avoid extensive harvest and harm at single place.

6.3. CONSERVATION PLAN

In the present study on the Yartsa gumba and it exploitation in India, the present author proposes the following steps for the conservation of this important natural recourses.

- Exploration of the presence of the fungus in different places of the Himalayan region.
- Area mapping of its occurrence and calendar of availability of Yartsa gumba.
- Documentation of occurrence and status of the wild population.
- Formulation of strategy for conservation as well as sustainable harvesting of the fungus.
- Training on larval stages of host insects to gatherers to avoid exposure and killing of uninfected caterpillar.
- Determination of route and area of collection year to year.
- Some groups monitoring agencies (CDRI, CIMAP etc.) must be involved in the purchase of harvested materials to maintain standard of Yartsa gumba.

6.4. MEDICINAL PROPERTIES AND BENEFICIAL USES

Cordyceps has a broad range of pharmacological and biological actions on the liver, kidneys, heart respiratory, digestive, glandular,

circulatory, metabolism and immune system which is due to its bioactive polysaccharides, modified nucleosides and Cyclosporine like metabolites produced by the fungus. (Yang Sang, 1989; Leu *et al.*, 1986; Cao, 1995; Chen, 1995; Han, 1995; Hsu *et al.*, 2003;Bao., 1995; Das *et al.*, 2005 and Wang, 1995).

Cordyceps may exhibit mild blood thinning properties so caution is to be taken while taking anti-coagulant. It enhances overall immunity by increasing the number of lymphocytes and natural killer cells and the production of interleukin, interferon and tumor necrosis factor. Recent studies, carried out at Beijing Medical University of China and Japan, have shown a 64% success rate among men suffering from impotence. In the ancient China, Cordyceps was highly recommended as one of the most effective medicines for all illness. Due to its anti aging and cure all properties, it can be compared to ginseng, reishi and deer velvet. In general Cordyceps is a tonic that help the body build strength, improve the organic functioning strengthen the immune system and bring longevity. At present this medicine has also a place in modern clinical practices. Zhu et al. (1998) reviews the Cordyceps effect of the fungus in various treatments (Table 10).

Chinese, Tibetan, Japanese and other many workers have reported its varieties of beneficial properties and medicinal uses (Table 10) since the ancient time.(Hobbs, 1998; Starnets, 1998;Cao and Yen, 1993; Leu *et al.*, 1986; Bao *et al.*, 1988; Bao,T.T. 1995; Bok *et al.*, 1999; Chen *et al.*, 1997; Chiou *et al.*, 2000; Halpern, 1999; Hsue and L.O., 2002; Huang, 1999; Ito & Hirano, 1997; Kuo *et al.*, 1996; Munzo, 1999; Wang-Shang *et al.*, 2000; Xie, *et al.*, 1988; Zhu, 1990; Li and Shen,2003; Cao and Yen, 1993 and Keho *et al.*, 1996).

Table 10. MEDICINAL PROPERTIES AND BENEFICIAL USES.

S.No.	Properties	Mechanism
1.	Blood Pressure Regulation	Low & High blood pressure by increasing nitric oxide and actually increase blood supply to heart and brain. Also slows down the heart rate and increase the blood volume of the heart. It also relaxes the blood vessel wall so blood flow is increased to the heart and blood pressure is lowered. Also helps lower to blood cholesterol and the low-density lipoproteins so common to heart diseases.
2.	Blood Builder, purifier and improvement of heart	Cordyceps increase blood cell viability and function and it is great blood builder and purifier. It clears anemia due to its ability to build bone marrow and platelet counts. Reduces phlegm and stop hemorrhages. Also helps normalize arrhythmia i.e. irregular heart beat and improve chronic heart failure.
3.	Regulation of Blood sugar and maintain cholesterol	Helps to regulate blood sugar Cordyceps is powerful natural anti infective agent. It helps in diabetes, cholesterol, triglyceride, LDL and raises HDL. It helps to lower total cholesterol by 10 to 21% and triglycerides by 9 to 26%. At the same time it helps to increase HDL- Cholesterol by 27-30% . Also decreases blood lactic acid.
4.	Regulate respiratory function and disease	Cordyceps alleviate the symptoms of several respiratory illness and improves the internal balance mechanism, making the utilization of oxygen more efficient. Reduces asthma and other respiratory problems to relieve and improve respiratory illness. It relax the bronchial walls as well as acting as an anti inflammatory agent. It is also effective against cough and phlegm, increases body adrenaline production which has natural anti asthma effect. Low adrenalines lead to symptoms of stress as well as eczema or other skin conditions.

5.	Increases Cellular Oxygen	It increases cellular oxygen absorption by up to 40%.
6.	Increases Energy	Cordyceps increases energy levels by increasing ATP production in the mitochondria.
7.	Protects Liver	Improves liver functions and helps in hepatitis and cirrhosis. It also cleans the blood and all other fluids of impurities. Chronic Hepatitis B patients found the extract increased their blood albumin levels as well as improving their liver and protein metabolism. It also improves bio-energy of the liver.
8.	Improves Kidney diseases	Improve chronic kidney diseases to 51% after only one month with Cordyceps supplement.
9.	Anti Aging Function	It is first anti aging supplement known to mankind and amazing anti aging herb Cordyceps help prevent the formation of lipid peroxides (age inducing free radicals) and can inhibits the formation of other free radicals in the brain.
10.	Protection Against Free Radical Damage	Several studies have shown that Cordyceps protects against the damages caused by free radicals and as such has powerful antioxidant properties.
11.	Natural Anti Cancer and Anti Tumor Agent	In China it is well known anti cancer, anti tumor and anti-infective agent. It has been shown significantly boost depressed immune function including both B and T cell lymphocytes. Studies have shown outstanding result with lung cancer and lymphoma patients. Research has shown that the Cordyceps has no effect outside the body such as in a test tube instead it works only inside the body by regulating the body's immune system to overcome diseases.
12.	Reduce Side Effects	It helps reduce the side effects of radiation therapy, such as used in cancer therapy.
13.	Increases Effectiveness of	Also increases the effectiveness of the spleen to help filter our harmful substances in the blood. It

	spleen	also helps to promote faster healing.
14.	Increases Absorption of the other Nutrition	Cordyceps makes other nutrients more effective when it is taken regularly, the food that is taken is fully absorbed.
15.	Improves Memory	It has a natural relaxing effect due to its amino acids, glutamic acid, tyrosine and L-tryptophan. It has also an excellent potential to benefit those with depression since it naturally inhibits monoamine oxidase in the brain with no side effects unlike drugs.
16.	Sex- Rejuvenator	It helps rejuvenate male sexual ability. Cordyceps is potent male sex hormones to bring back youthful function and stamina.
17.	Restful Sleep	It has calming effect on the nervous systems to help reduce anxiety and nervous stress as well as making sleep more sound.
18.	Genetic Improvement	It promotes genetic repair.
19.	Blood Supply	Increase blood supply to heart and brain.
20.	Balancing and normalizing effect on human physiology	Cordyceps promotes both the 'Yin and Yang' aspect of the body thus it has a very balancing, normalizing effect on many facets of human physiology.
21.	Acts Adaptogen	It is an adaptogen that may be useful in increasing endurance improving general health and increasing testosterone levels.
22.	Cures	Cough, anemia, back pain, impotence, infertility, irregular menstruation, night sweats, senile weakness, soreness of loins and knees, dizziness, tinnitus, osteoporosis, hypothyroidism cancer, nephritis and stomach ailments.
23.	Improve Appetite	
24.	Promote Good Health and Vitality	

25.	Cures Opium a Addiction, Poisoning, expectorant and Anti asthmatic	
26.	Useful for weak back and knees, Impotence	
27.	Promote phagocytosis	
28.	Enhance the Function of the Reticuloendothelial system.	
29.	Cures Insomnia Anxiety, Adrenal Hypo function	
30.	Best for anyone who leads a very busy life and needs extra energy	
31.	Anti oxidant (Scavenger free radicals)	
32.	Used as Tranquilizer or sedative in TCM	

6.5 IMPACT ON SOCIO–ECONOMY

During the field study of Yartsa gumba it was found families of 24 villagers economically depend on Yartsa gumba trade were contacted to share their experience, views to assess their livelihood and socio economic status and sources of their income. Total population of 24 villages is 2363 out of which male and female are 1231 (52.09%) and 1132 (47.90%) respectively. Further out of these, Schedule Tribes and Schedule Caste are 408 (17.27%) and 1000 (42.32%) respectively. During investigation it was noticed that entire population 1408 of Schedule Tribes and Schedule Caste use to collect Yartsa gumba as an alternate source of income during short summer period. Main source of income of the people of the areas is a multidisciplinary approach by practicing cultivation of vegetables, rajmash production in their fragmented small holdings in hilly terrain, maintenance of small herd of sheep and goat for wool and meat production and newly inducted German Angora rabbit for wool production. In recent past some people also use to visit to high altitude areas to collect medicinal and herbal plants for their revenue generation. The poor income due to small holding and other meager sources force the local people to migrate to plain areas in search of jobs and this has been practiced for a long time. However, the discovery of Yartsa gumba a high medicinal value produce in recent past has opened a new avenue for the unemployed youth.

Migration in high altitude along with their herd (Fig.24 and 25) is also a practice of local people where they use to grow some short duration vegetables crop and with the onset of winter they further migrate down to their main habitat. Thus the locals earn their livelihood from different sources. In present scenario during May to mid July locals go to the areas of natural habitat of Yartsa gumba (Fig.26) and keep keen watch and start collection. This short term collection of Yartsa gumba is more

paying as compared to their traditional year long agri-horticulture and animal venture besides fabrication of wool products like, Shawl, Tholma, Carpet, Dun, Pankhi etc. Moreover market of Yartsa gumba is very fast and there is no delay in disposal of high value medicinal raw material as compared to their traditionally prepared articles.

It has been observed that Yartsa gumba being sporadic and scattered in nature is not so easily visible to the eyes. Local sits on the grounds and sometime they lie on the ground and spot out stalk (Fig. 27 and 28) which is more or less green grass blade like structure and almost camouflage with the grasses of the area. However, its peculiar structure differs. Local people dig out the specimen keeping in view to avoid breakage of stalk and put in aluminum plates (Fig. 29 and 30) or baskets. It has been recorded that after rigorous collection, one skill person can collect 30-40 specimen of Yartsa gumba per day, however, quantity of collection also depends on the locations, occurrence and abundance of the specimen. Collection of Yartsa gumba has opened a new source of avenue for the revenue generation of the locals and their socio economic improvement accordingly. It was observed that during digging number of uninfected larval/pupal stages are either exposed or damaged because of the ignorance of gatherers. However, a few of them are aware and they further keep such stages safely under the soil and show keen interest to conserve it for further harvesting. In present study it was observed that a skill person collecting 30-40 specimen earn Rs.400-500 per day after strenuous efforts without wasting his time. Thus in one season of 60 days Rs.17000-25000 can be earned by one skill person. However, one family consisting of 5 members of different age groups is able to earn Rs.75000-120000 in a season of the year and this amount is adequate for their livelihood without migrating to plain The present findings supports the observation of Arif & Kumar, 2003; Das *et al.*, 2005; Negi, 2003 a, b and c; 2006, Negi *et al.*, 2003; Garbyal *et al.*, 2004 and Sharma, 2004.

Table 9. Population of 24 villages relying on Yartsa gumba.

S. No.	Name of village	Total Population	Male	Female	Schedule Tribe			Schedule Caste		
					Total PPL	Male	Female	Total PPL	Male	Female
1.	Milam	37	28	9	5	5	-	29	20	9
2.	Bilchhu	11	9	2	3	3	-	7	5	2
3.	Panchu	30	21	9	1	1	-	29	20	9
4.	Ganghar	9	5	4	1	1	-	8	4	4
5.	Mapa	10	7	3	-	-	-	10	7	3
6.	Burfu	55	37	18	23	16	7	29	18	11
7.	Tola	20	9	11	6	3	3	14	6	8
8.	Martoli	25	21	4	7	4	3	18	17	1
9.	Lua	7	7	-	1	1	-	5	5	-
10.	Laspa	19	14	5	3	3	-	16	11	5
11.	Khilamb	19	9	10	15	6	9	4	3	1
12.	Ralam	102	50	52	2	1	1	100	49	51
13.	Relkot	10	10	-	-	-	-	10	10	-
14.	Pato	245	117	128	46	20	26	144	64	80
15.	Bui	279	151	128	57	28	29	138	78	60
16.	Lilam	45	25	20	-	-	-	21	12	9
17.	Saipole	366	182	184	235	119	116	73	34	39
18.	Saibhat	312	155	157	1	1	-	-	-	-
19.	Zimipa	149	66	83	-	-	-	146	64	82
20.	Kwiti	154	68	86	-	-	-	154	68	86
21.	Dhilam	164	84	80	2	2	-	7	3	4
22.	Kulpam	160	82	78	-	-	-	24	12	12
23.	Ugrarali	12	7	5	-	-	-	24	12	12
24.	Phalyati	123	67	56	-	-	-	-	-	-
Total		2363	1231	1132	408	214	194	1000	522	478
Percentage (%)			52.09	47.90	17.27	52.45	47.55	42.32	52.2	47.8

Chapter-7

Mass Production of Yartsa Gumba

MASS PRODUCTION OF YARTSA GUMBA

7.1. MATERIALS AND METHODS

Live specimens of Cordyceps were carefully collected from their natural habitat in the high altitude region (Laspa areas) at an altitude of 13000 feet during May and June 2004. The specimens were wrapped inside the moss plants and then packed in the ice cubes. The specimens were washed with tap water to remove the adhering dust particles on it. The stroma of the fresh specimen of Cordyceps were washed 2-3 times in double distilled water and dipped in 0.1% HgCl solution for one minute. Further the stroma was washed with sterile distilled water, surface dried by pressing between sterilized filter paper. In order to propagate the mycelium in vitro, tissues were taken from the different parts of the Cordyceps body like spores, stalk tissue and tissue from stroma region. These tissues were excised from the Cordyceps body with the help of a sterilized scalpel inside a laminar flow and cultured in to the various culture media. Eight different types of media were prepared to get the pure culture of the fungus. Culture media utilized for pure mycelium culture were Potato Dextrose Agar (PDA), Casein Hydrolysate Dextrose Agar (CHDA), Beef Extract Dextrose Agar (BEDA), Soyabean Seed Extract Dextrose Agar (SEDA), Rice Extract Dextorse Agar (REDA) and Black Soya Seed Extract Dextrose Agar (BSEDA). The chemical composition of each culture media is given in Table 11. pH of media varied from 4.5 to 6.5. The cultures were incubated at the various range of temperature (5 to 25^0C) inside the incubator.

7.2 RESULTS

Observations were taken for the mycelium spread on the different media. Results are shown in Fig. 54. It was observed that the tissue taken from the stroma region of the Cordyceps is the most suitable inoculum to get the mycelia run in the culture media. However, spores and stalk tissue did not response at all. Out of the 8 culture media the mycelial growth was successful on 5 culture media viz., PDA, BEDA, CHDA, SEDA and REDA .During the experiment it was also observed that the optimum growth of the Cordyceps occurred under low temperature condition between 5 to 15^0 C and more acidic pH 5-5.5 (Fig. 53). However, sclerosis was observed in the mycelium obtained on all the types of the culture media. With the result mycelia having a numerous spores were observed under the compound microscope (Fig. 51 and 52).

7.3. DISCUSSION

The availability of Yartsa gumba is scanty in nature and it involves a high labour cost to collect from its natural habitat as wild harvest. Under such circumstances, laboratory culture of this fungus is the only solution to fulfill the demand of such a high value medicinal and highly priced fungus. Hence the standardization of laboratory culture technique of present investigation of the fungus needs to be the prime importance. Thus laboratory production of the mycelium of Cordyceps will definitely prove a great success in preparation of various products from the dried mycelium which has numerous potential therapeutic applications.

In the early 1970's Chinese government promoted cultivation of over 200 species of wild Cordyceps, looking for the best type, finally isolated and selected *C. sinensis,* after conducting many studies using scientific standards to verify the safety and better medicinal properties of *C. sinensis*. The product of Chinese strain is of artificial medium are

commercially available in USA and Canada. The medicinal properties of fermented mycelium products have been examined in experimental and clinical trials which showed promising results. In Korea an association of mushroom biologists and mushroom growers is providing knowledge to farmer on Cordyceps fruiting body inoculation on synthetic media. (Zhu, *et al.*, 1990, Ikumoto, 1991, Manbe *et al.*, 1996, Kiho, 1996, Yamaguchi, *et al.*, 2000, Lie, *et al.*, 2001 and Zhao, *et al.*, 2002).

In India biotechnological study and extension service for its culture is badly needed .The present author suggest following steps for the benefit of the local people.

- Screening natural population of Cordyceps for its constituent.
- To develop a protocol for growing Indian strain in artificial medium.
- To compare the chemical composition of in vitro grown culture with naturally occurring Cordyceps for its quality.

Since wild Cordyceps is rare and difficult to harvest due to its growing in harsh environment as location and season specific efforts have been made to cultivate Cordyceps mycelia for commercial application. Commercial cultivation of Cordyceps began in the early 1980's making the herb readily available for clinical research. The active ingredients of CS-4 strain of C. sinensis are quite different (Zhao *et al.*, 2002; Zhou *et al.*, 1990 and Li, S.P. *et al.*, 2001).

1) Liquid culture of *C. sinensis* is a common practice in China. The fermentation in which the organism is introduced into a tank of sterilized liquid medium, which has been formulated to provide all the necessary nutritional components for rapid growth of the mycelium. After the growth in the liquid medium, the mycelium is harvested by straining out from the liquid broth and drying, after which it can be used for further processing. In this method the extra

cellular compounds, which were exuded by the fungus during the growth period are discarded with the spent broth. Thus causes a major loss of bioactive compounds as many of the active ingredients are extra cellular in nature and are found only in small concentrations in the mycelium.

2) America and Japan are practicing the solid-substrate cultivation method. In this system the mycelium is grown in plastic bags or glass jars containing sterilized medium, which contain some type of cereal grains viz., rice, wheat or rye. After some period of growth the mycelium is harvested along with the residual grains. This method is based on low capital investment cultivation technique and is easy for the growers. The down side of this method is that the grain content is usually greater than the mycelium content. However in this method the extra cellular compounds are harvested along with the substrate and mycelium. The compound cordycepin is primarily extra cellular in nature. The tests have shown the presence of cordycepin in solid substrate grown Cordyceps and absent in liquid cultured Cordyceps. Recently (Zhou *et al.*, 1990; Kiho, 1996; Yamaguchi *et al.*, 2000; Lie *et al.*, 2001 and Zhao *et al.*, 2002) have obtained Hydroxy Ethyl Adenosine (HEA) from a new hybrid Cordyceps after cross breeding by advanced techniques, the wild strain of *C. sinensis* occurring at an altitude of 21000 feet on the snow covered peaks of Himalayas for the quality of product and *C. sobolifera* occurring in the low bamboo forest of China. Continuous efforts have been made to increase concentration of HEA and cordycepin the compound much in demand worldwide. The improved techniques available to the growers has resulted in an increase of 1500%

higher HEA and cordycepin yield. Further the products are pure and without contamination of soil particles.

Zhou *et al.* (1998) and Hobbs (1986) reported that genus *Cordyceps* produce some potent antibiotics. Further, these authors doubted whether the species of genus *Cordyceps* are single organism or they are symbiotic colonies of more than one organism. They doubt that todays *C. sinensis* will one day be known as a fungal/bacterial symbiosis. However, DNA sequencing is inconclusive in this regard as the DNA sequence tends to change with time, as if the fungus were incorporating some of the insect DNA into its own DNA code for the initiation of its fruiting body form, then loosing the insect DNA when it goes back into its mycelial form, microscopic examination of growing *C. sinensis* mycelium reveals some very interesting morphology including the concurrent anamorphous of filamentous mycelium and rapidly moving single celled yeast like morphological form. This has been observed in other *Cordyceps* spp. as well such as *C. sobolifera.*

Thus the culture method itself has an effect on the quality of the resultant Cordyceps product. Besides methodology the next factor in the production of particular secondary metabolized or target medicinal compounds is dependant on the nature and composition of the substrate itself. Further a substrate which favours rapid and strong growth of the mycelium would be an ideal substrate for use (Zhang *et al.,* 1992). The culture medium for the development of *C. sinensis* is in progress (Fig. 54). The chemical analysis of initial products is *in-vitro* is in progress (Table. 11). Further studies are in progress.

Table 11. Chemical composition of different culture media.

Constituents	Media (g/ liter)							
	PDA	CHDA	BEDA	SEDA	REDA	MEDA	SBEDA	BSEDA
Peptone	10	-	10	-	-	-	-	-
Dextose	40	40	40	40	40	40	40	40
Casein Hydrolysates	-	10	-	-	-	-	-	-
Beef extract	-	-	3	-	-	-	-	-
Sodium Chloride	-	-	5	-	-	-	-	-
Mushroom Powder	-	-	-	-	-	50	-	-
Soyabean powder	-	-	-	80	-	-	-	-
Rice Powder	-	-	-	-	100	-	-	-
Black Soya Powder	-	-	-	-	-	-	-	80
Soya been Powder	-	-	-	-	-	-	40	-
Agar Powder	15	15	15	15	15	15	15	15

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Figures

FIGURES

Fig: 1 Route map of expedition to study area.

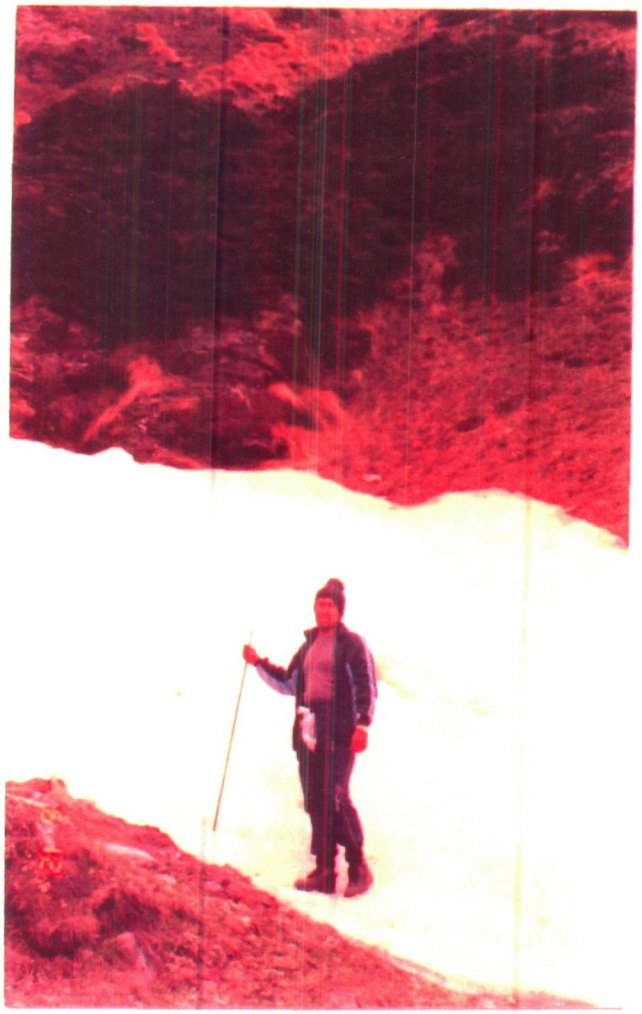

Fig: 2 Trekking in high altitude areas snowbound for collection and study.

Fig: 3 Trekking in high altitude areas for collection and study.

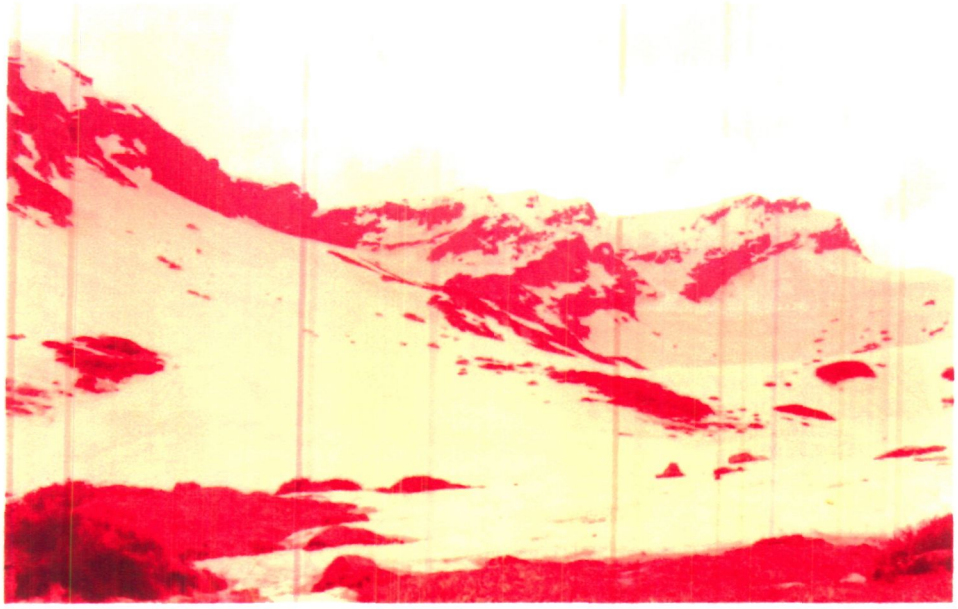

Fig: 4 Area of occurrence of Yartsa Gumba after snow melt.

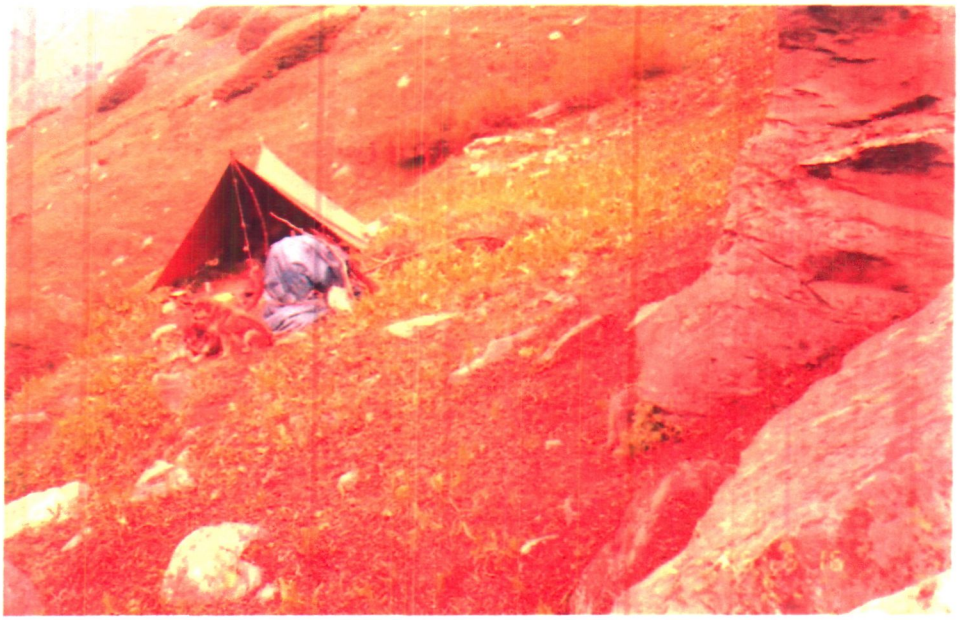

Fig: 5 Camping at 12000 feet altitude for collection and study.

Fig: 6 Camping at 15000 feet altitude for collection and study.

Fig: 7 Melting of snow–clearance of habitat of Yartsa Gumba.

Fig: 8 Vegetation at 14000 feet as host of *Hepialus*.

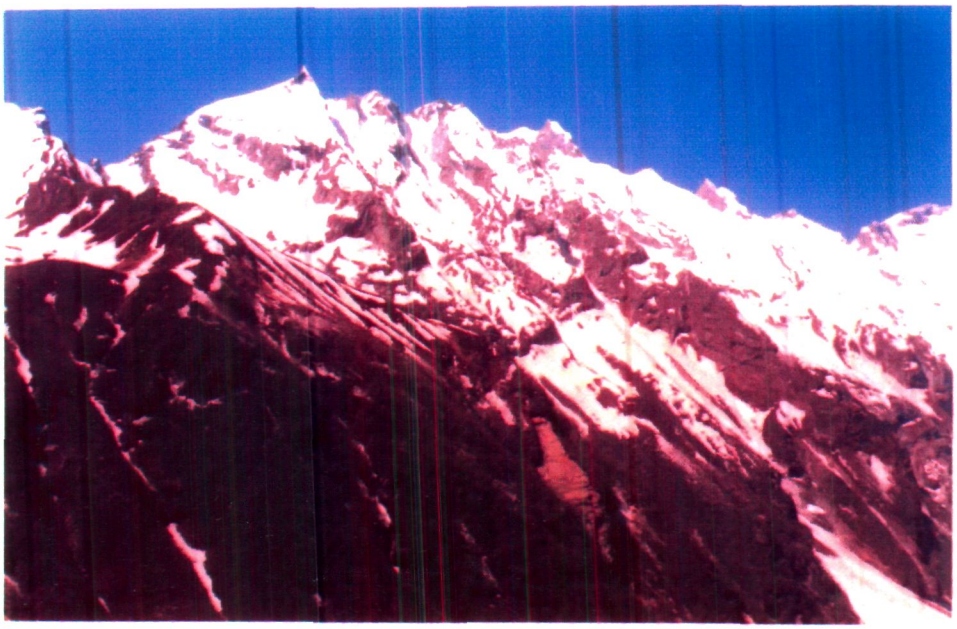

Fig: 9 Occurrence of *Hepialus* at 14000 feet altitude- on slope.

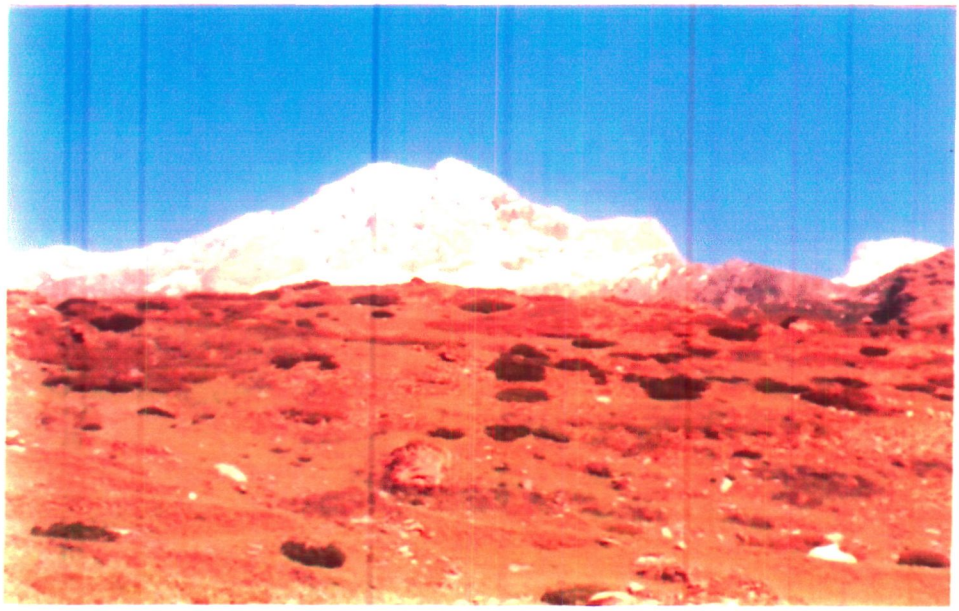

Fig: 10 Habitat of Yartsa Gumba with vegetation after snow melt.

Fig: 11 Camp for survey and collection of Yartsa Gumba at 13000 feet.

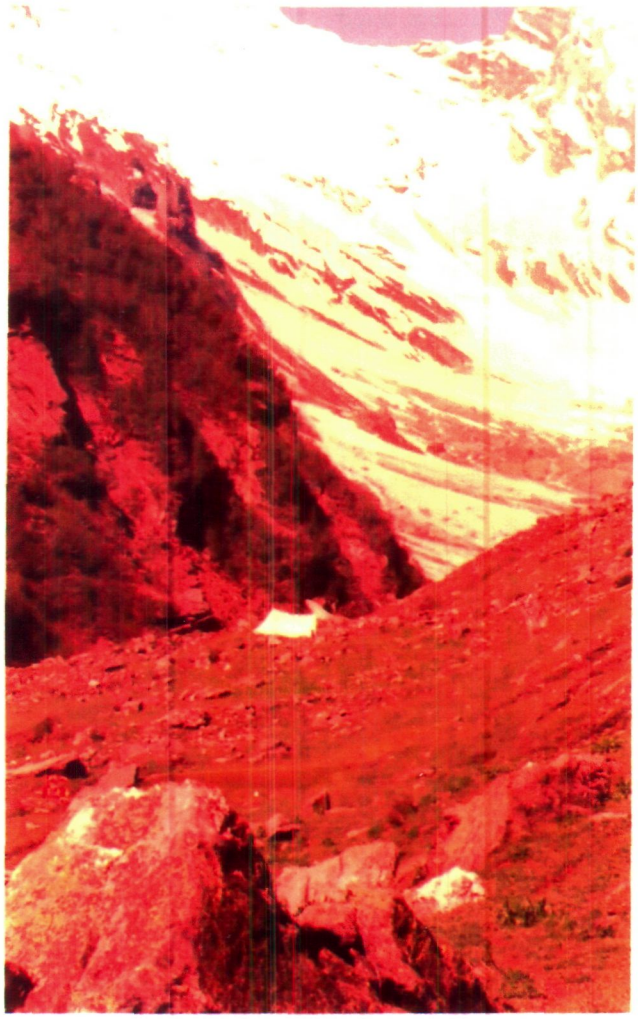

Fig: 12 Camping at 14000 feet altitude for collection and study.

Fig: 13 Occurrence of Yartsa Gumba at 14000 feet altitude.

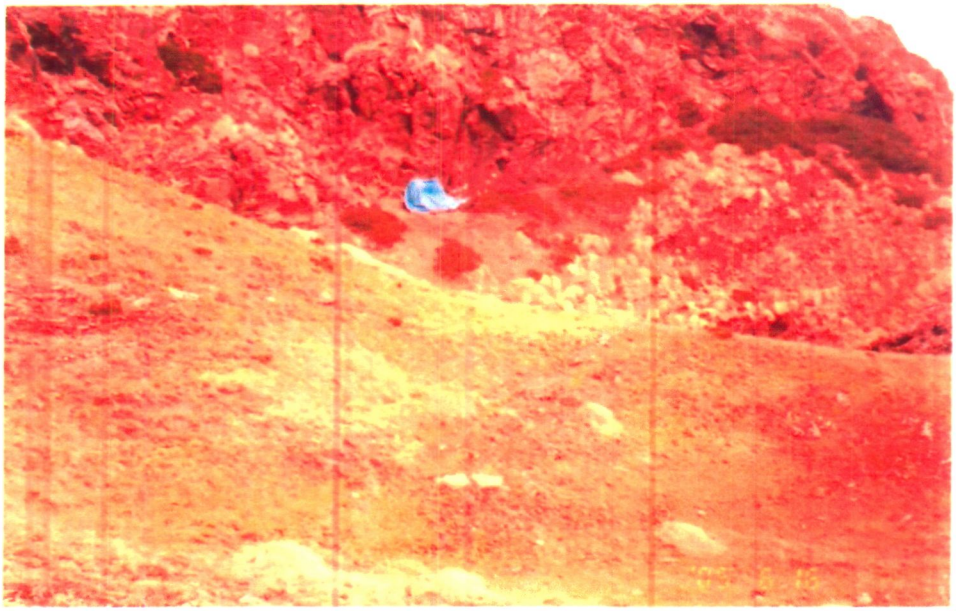

Fig: 14 Camping at 15000 feet altitude.

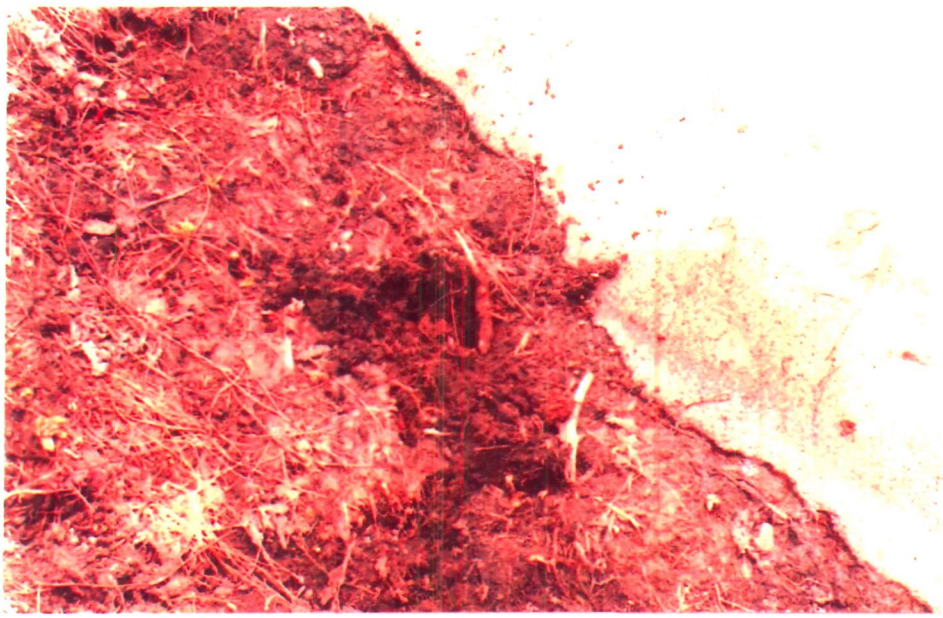

Fig: 15 Yarsta Gumba in nature – just after snow melt.

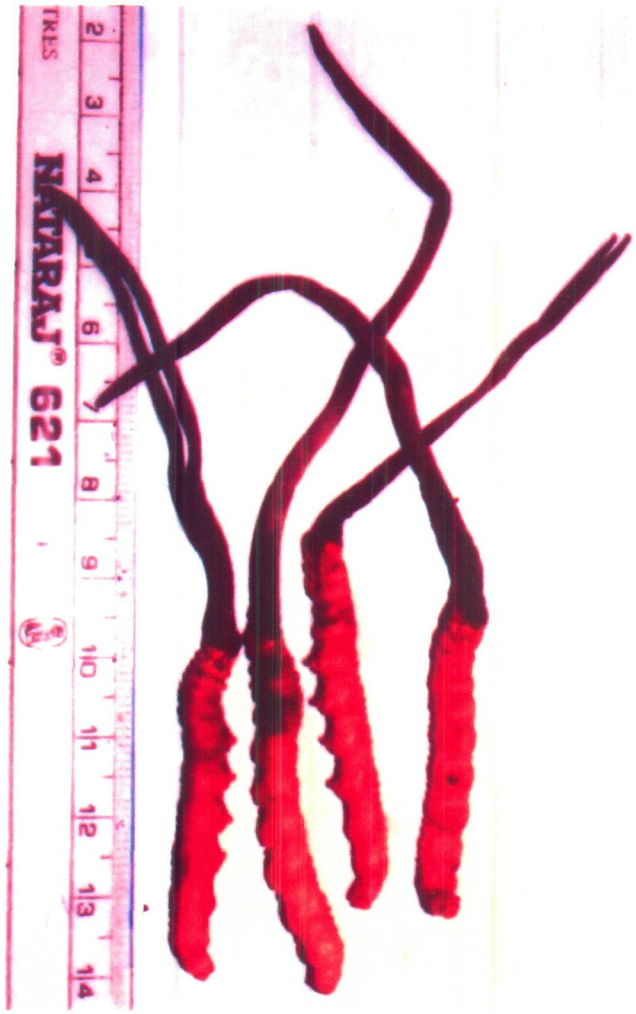

Fig: 16 Fifth instar larva of 5 cm with 10 cm stalk and specimen with double stalk.

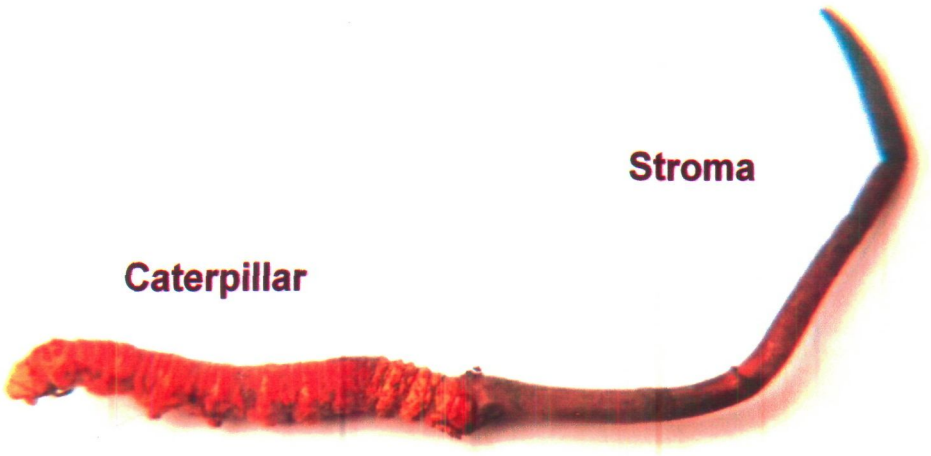

Fig: 17 Magnified caterpillar with stalk.

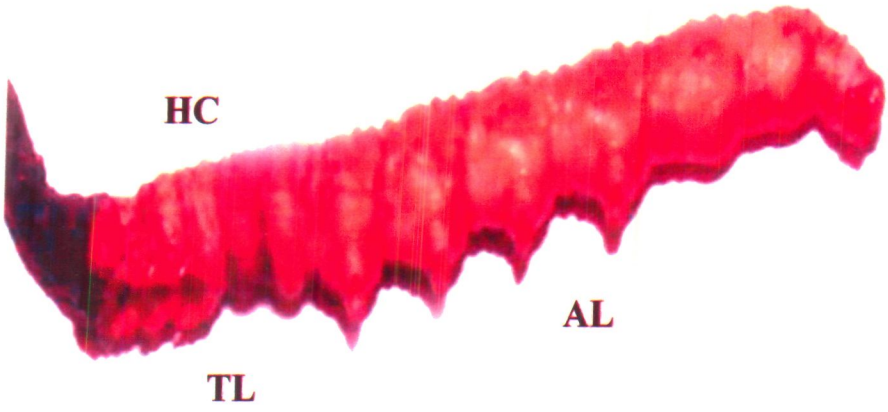

Fig: 18 Magnified caterpillar (HC-Head Capsule, TL-Thoracic leg and AL-Abdominal legs).

Fig: 19 Yartsa Gumba with double stromata.

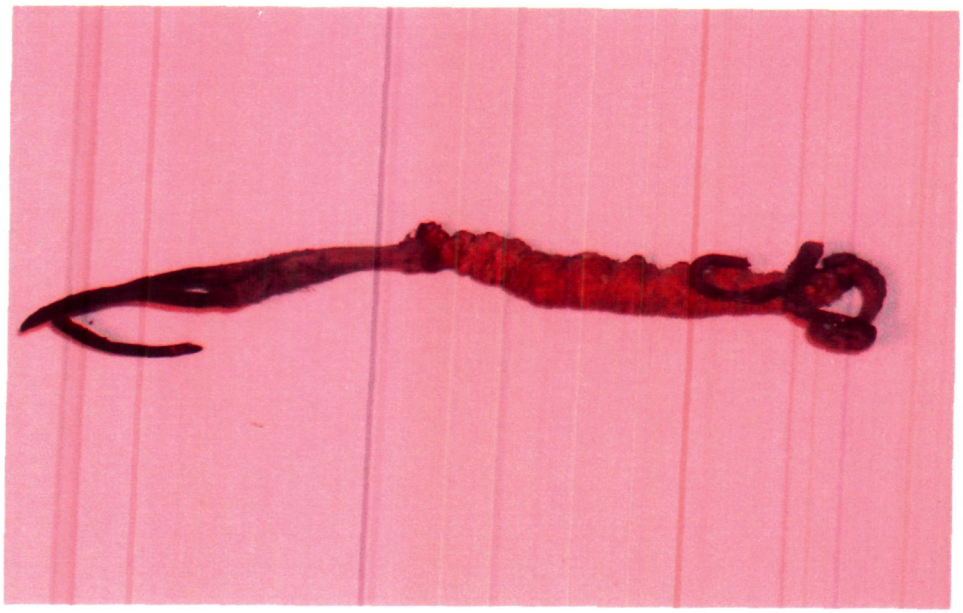

Fig: 20 Specimen with double sided stromata –A peculiar Yartsa Gumba.

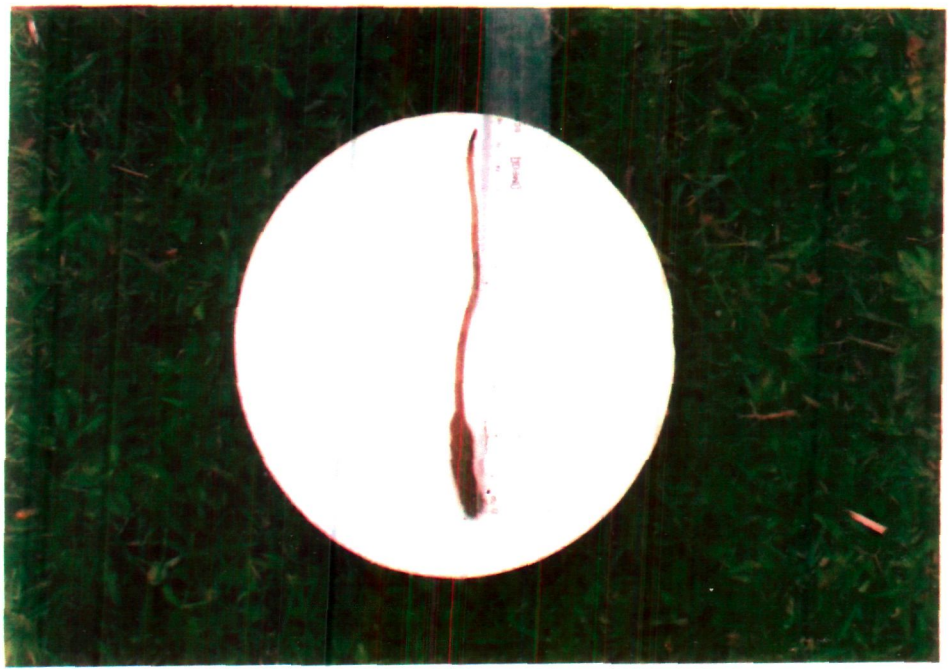

Fig: 21 Abnormal size of caterpillar with stalk measuring 16 cm (5 cm caterpillar and 11 cm stalk).

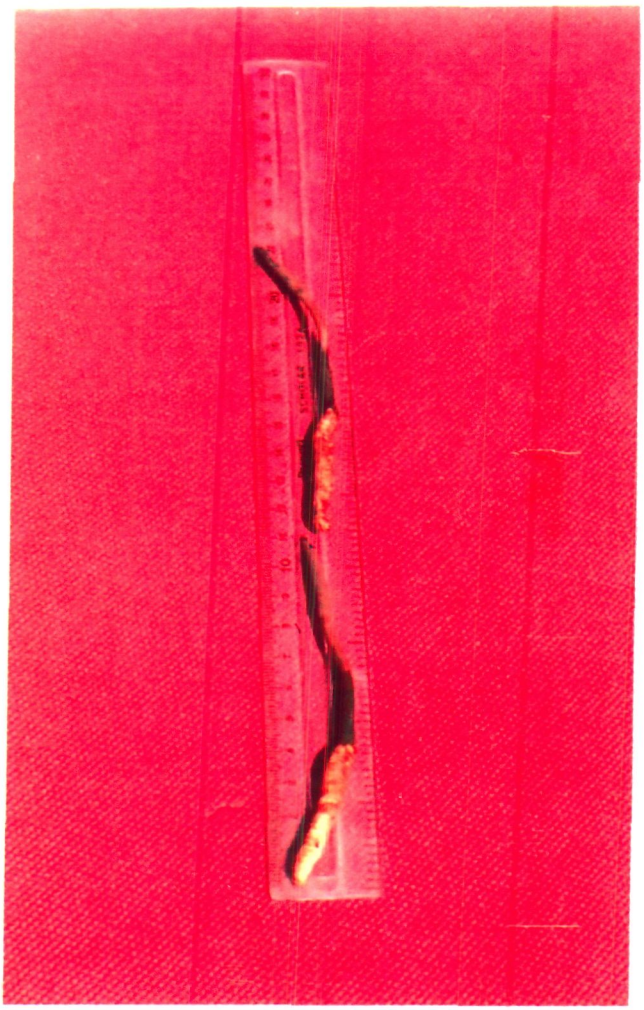

Fig: 22 Measurement of freshly dug out caterpillar with stalk of different sizes.

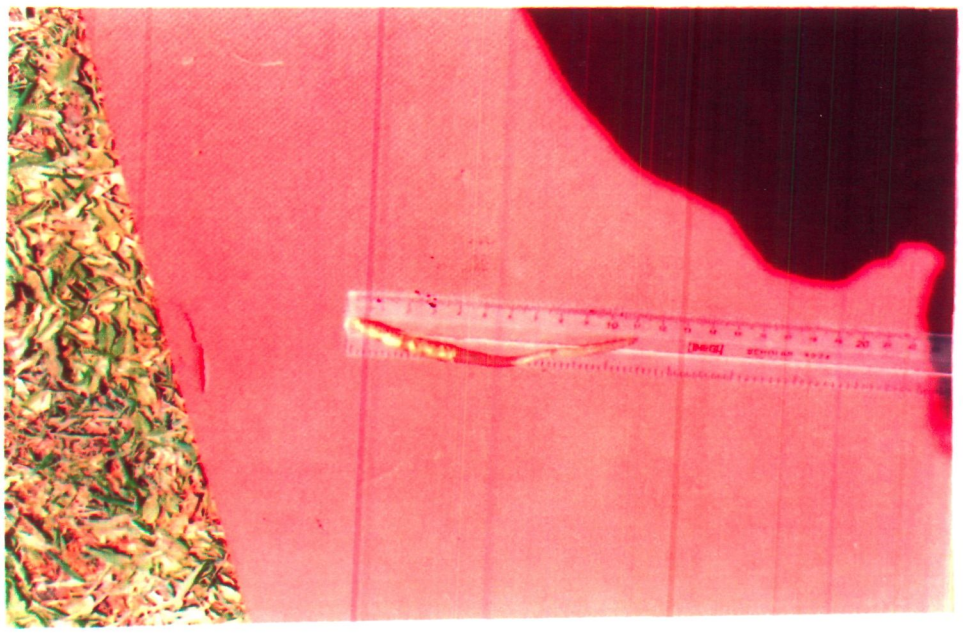

Fig: 23 Caterpillar with stalk measuring 11 cm (5 cm caterpillar and 6 cm stalk).

Fig: 24 Villagers moving with their sheeps upwards for collection of Yartsa Gumba.

Fig: 25 Villagers moving up with full preparation for collection of Yartsa Gumba.

Fig: 26 Men and Woman of village of Munsiyari trekking for collection of Yartsa Gumba.

Fig: 27 Digging of Yartsa Gumba in its habitat.

Fig: 28 Close view of digging of Yartsa Gumba.

Fig: 29 Yartsa Gumba-collected by villagers and drying in open.

Fig: 30 Yartsa Gumba- freshly collected from soil, stored in aluminum plate.

Fig: 31 Freshly dug out Yartsa Gumba.

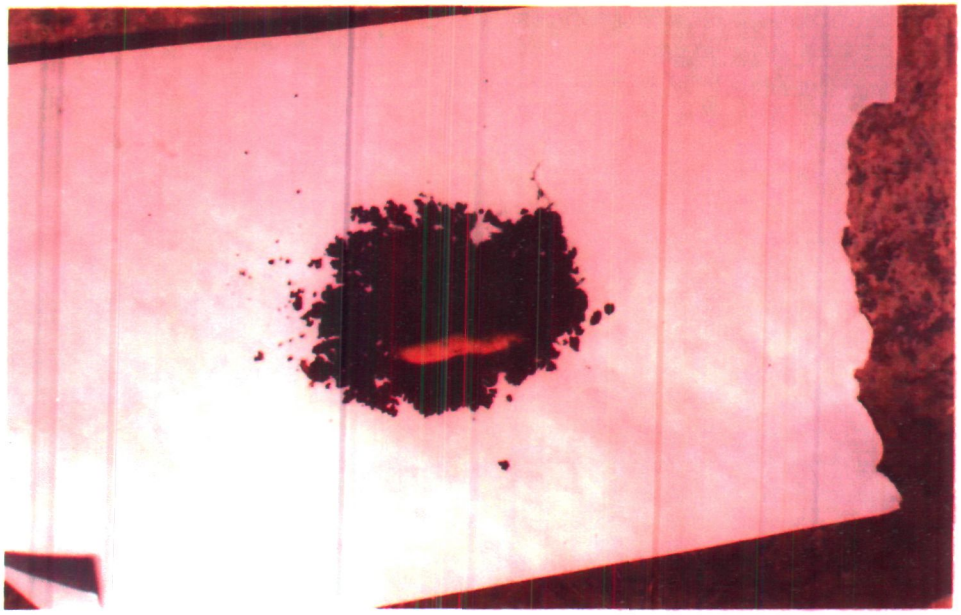

Fig: 32 Live caterpillar of *Hepialus* dug out of the soil.

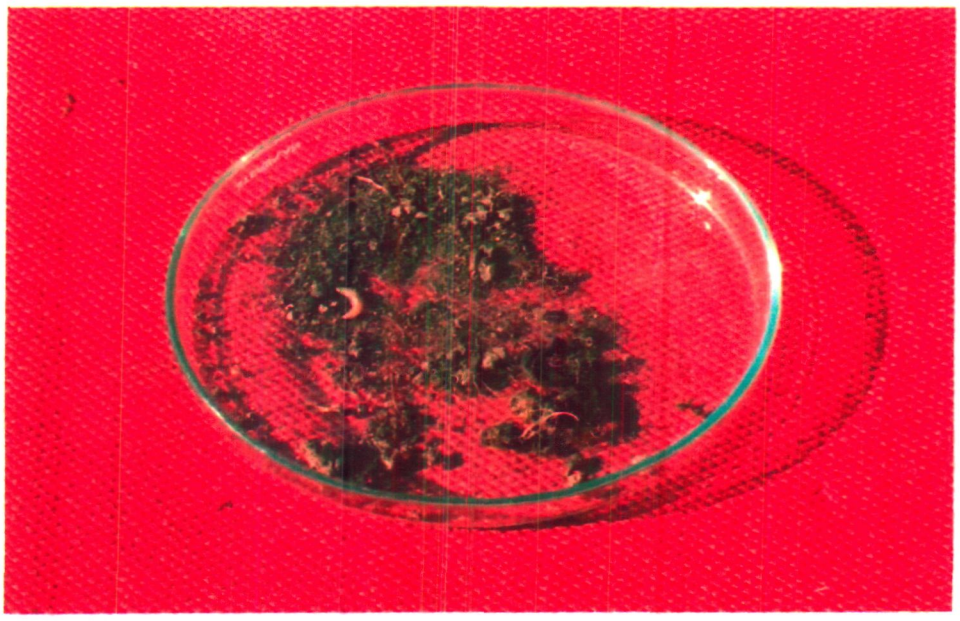

Fig: 33 Second instar larva of *Hepialus* with soil.

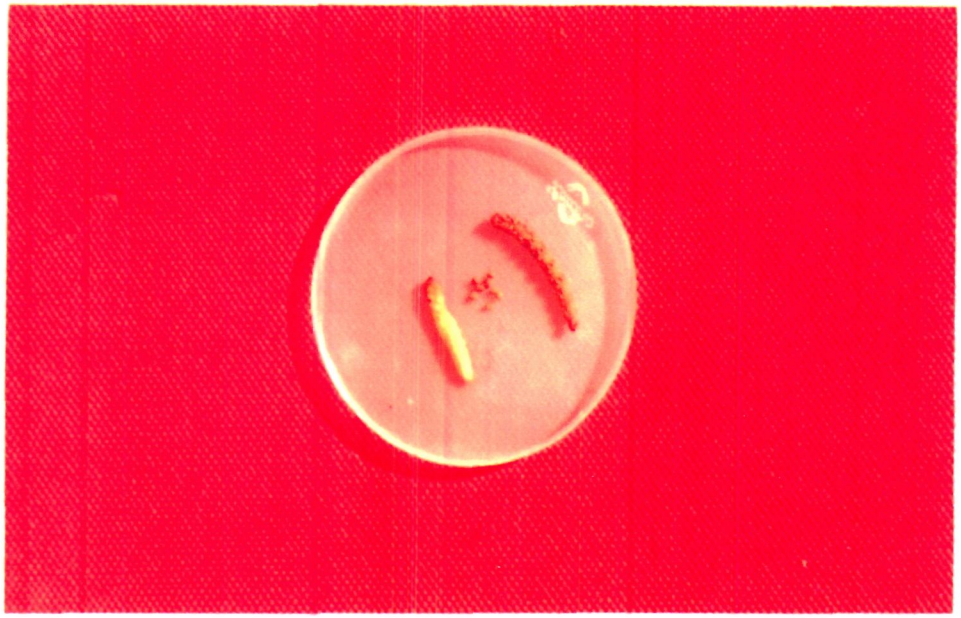

Fig: 34 Third and fourth instar larva of *Hepialus*.

Fig: 35 Different instars of *Hepialus* dug out of the soil.

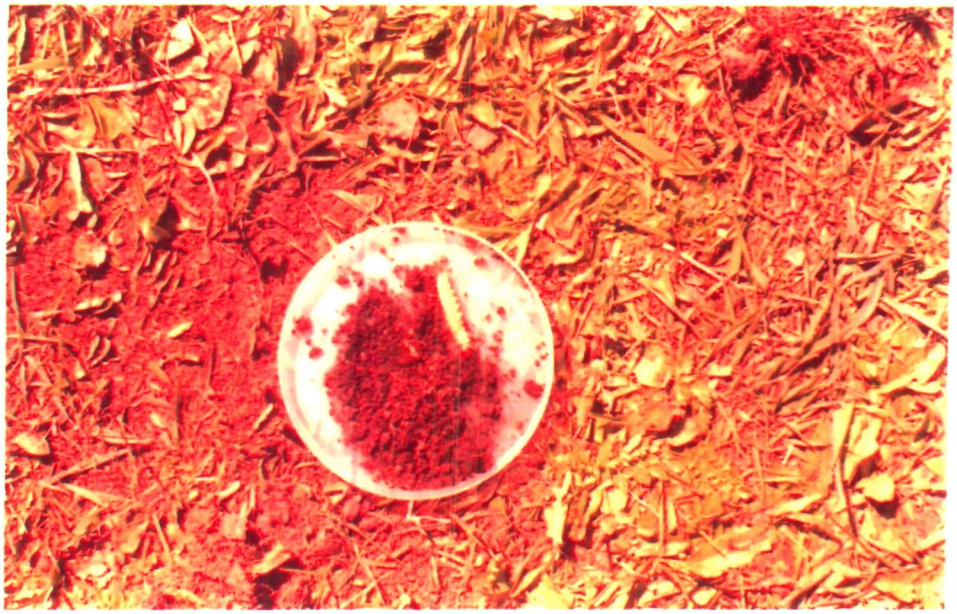

Fig: 36 *Hepialus* caterpillar dug out from its habitat.

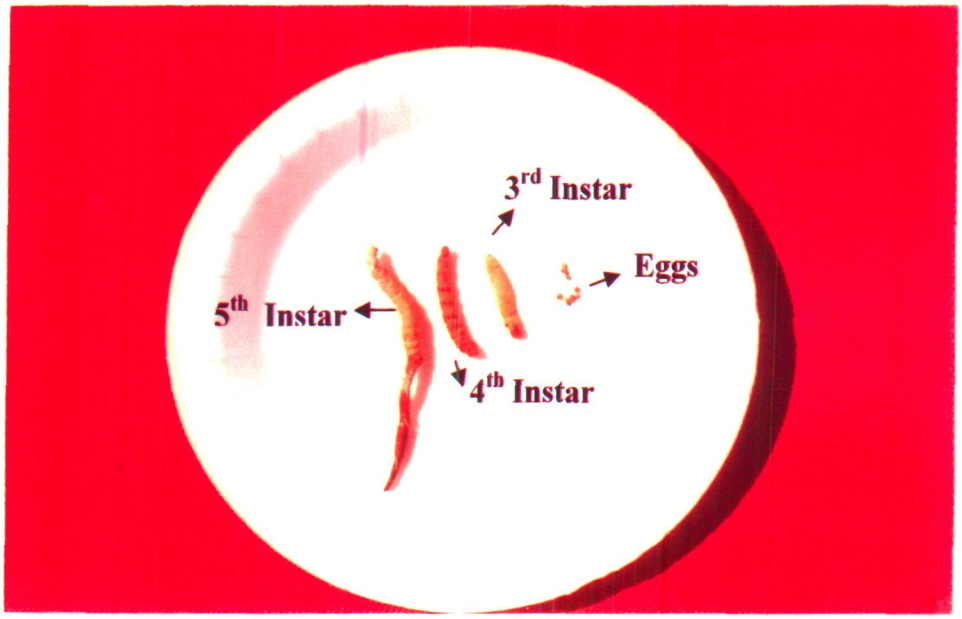

Fig: 37 Egg 3rd, 4th instar (uninfected) larva and 5th instar (infected) caterpillar.

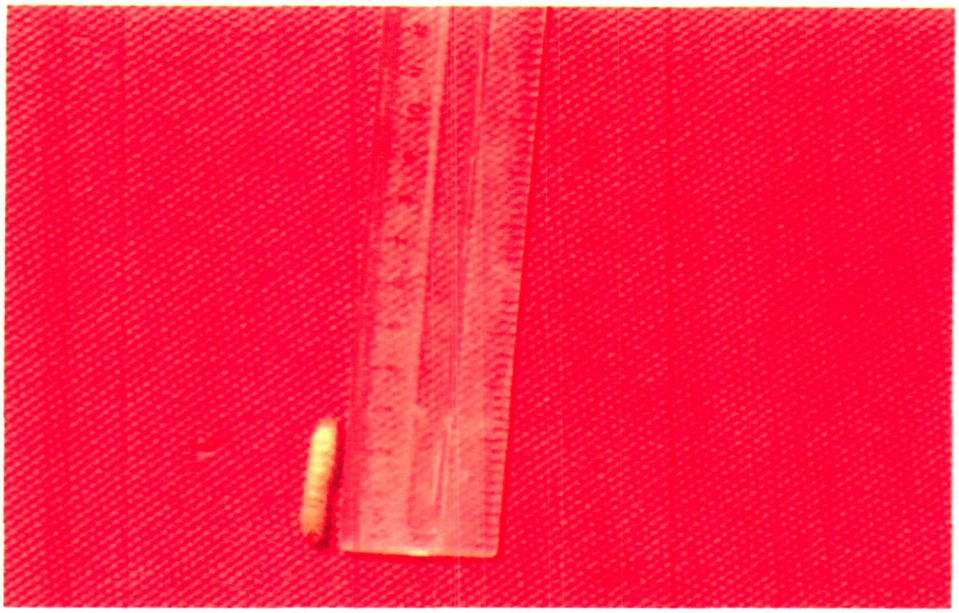

Fig: 38 Freshly collected fourth instar larva of *Hepialus.*

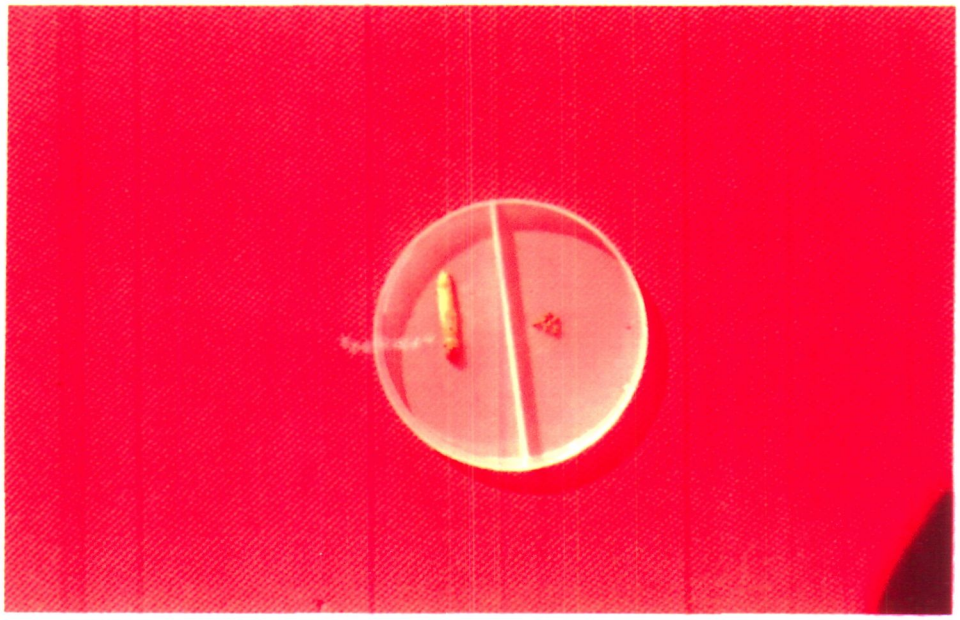

Fig: 39 Fourth instar larva and eggs of *Hepialus*.

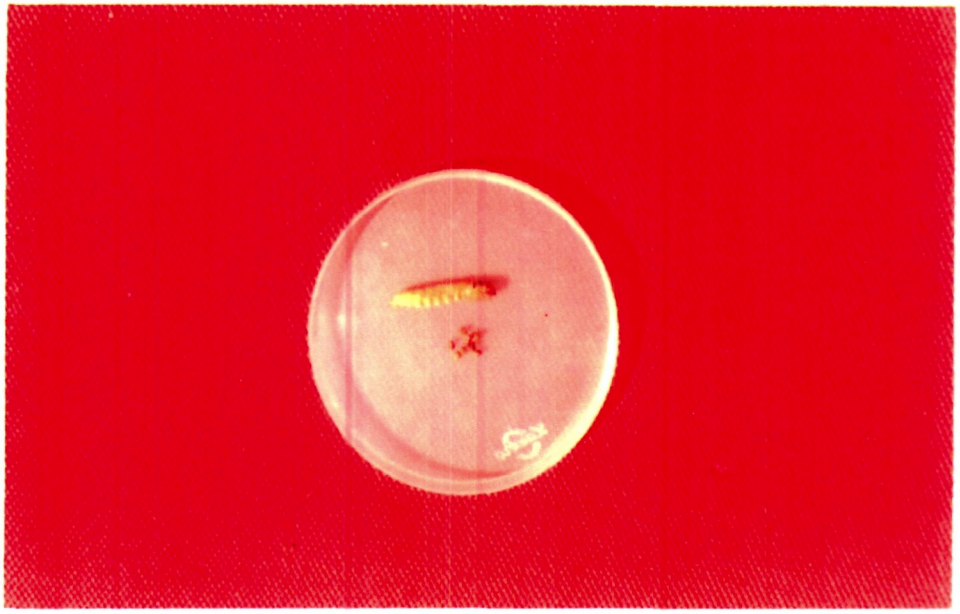

Fig: 40 Fourth instar larva of *Hepialus* and eggs collected from host plants.

Fig: 41 Pupa of *Hepialus* collected fresh.

Fig: 42 Pupa of *Hepialus.*

Fig: 43 Pupal formation of *Hepialus* (dug out from 14-20 cm).

Fig: 44 Pupal formation of *Hepialus* (naturally).

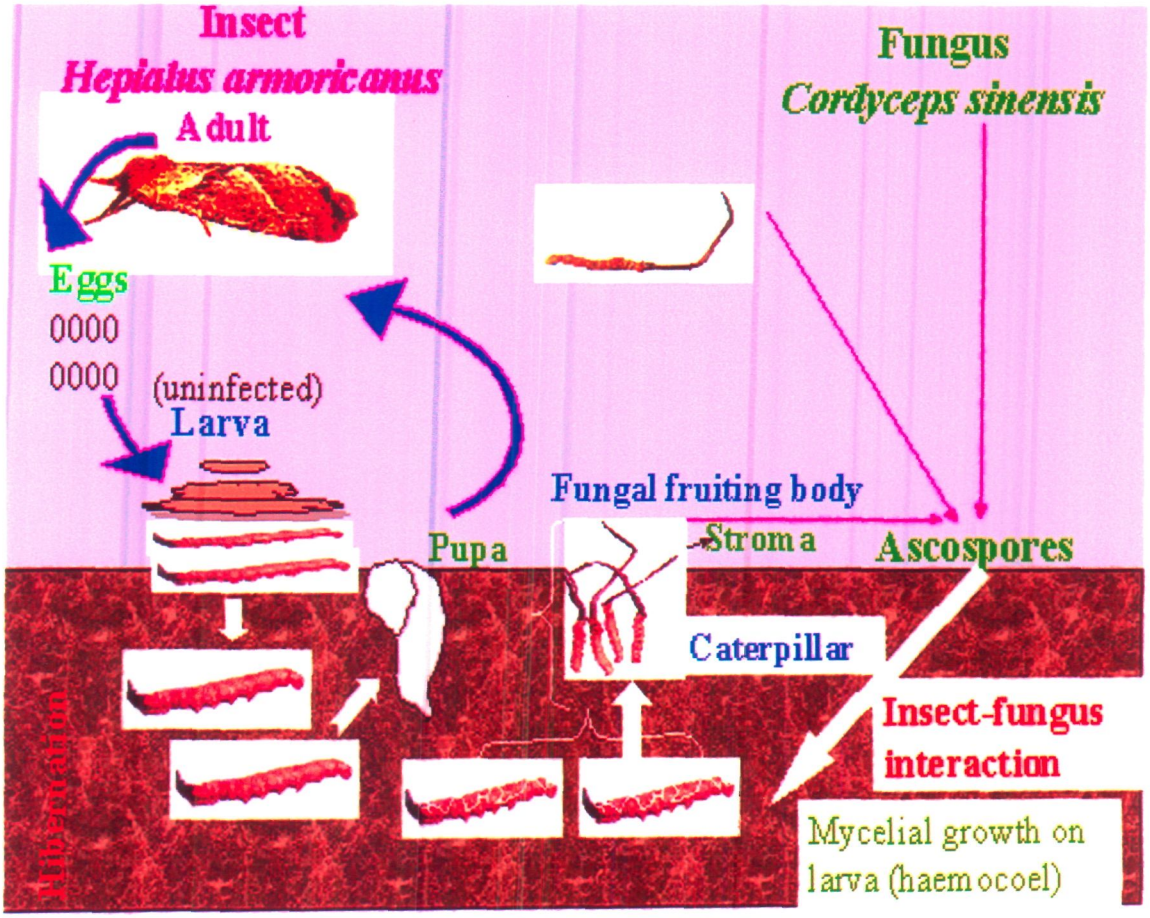

Fig. 45 Life cycle of *Hepialus armoricanus*- sketch.

Fig:46 Yartsa Gumba with double and four stalk-an variation.

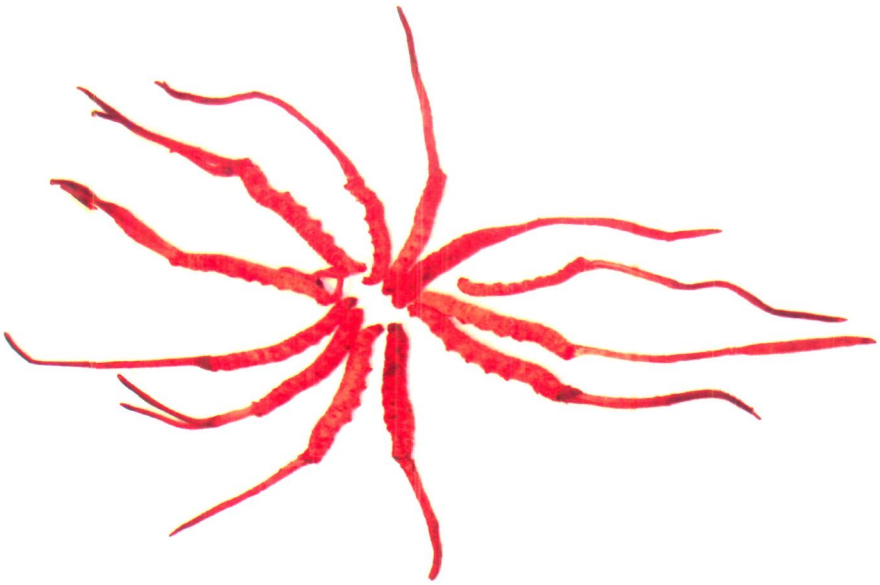

Fig: 47 Variation in size and stalk of Yartsa Gumba.

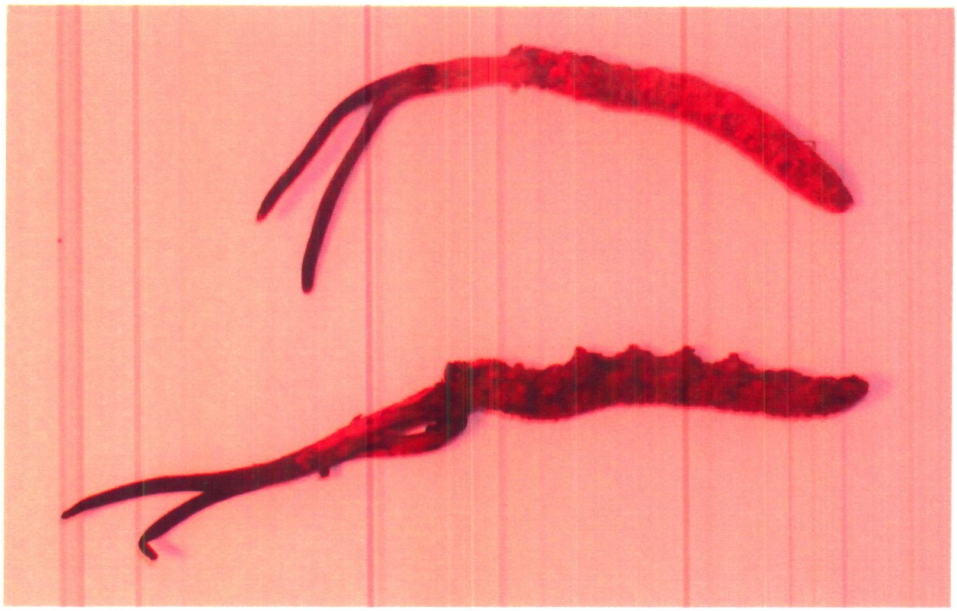

Fig: 48 Variation in double stroma of Yartsa Gumba.

Fig: 49 Variation in size of Yartsa Gumba and its club shaped stroma.

Fig: 50 Wild collection of Yartsa Gumba.

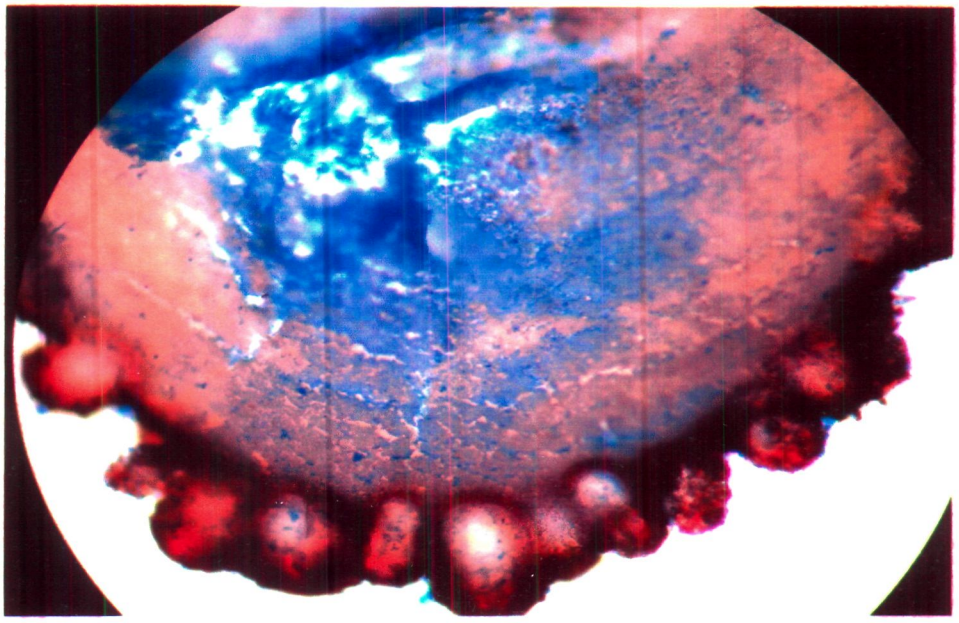

Fig: 51 T.S. of stroma showing peritheca attached.

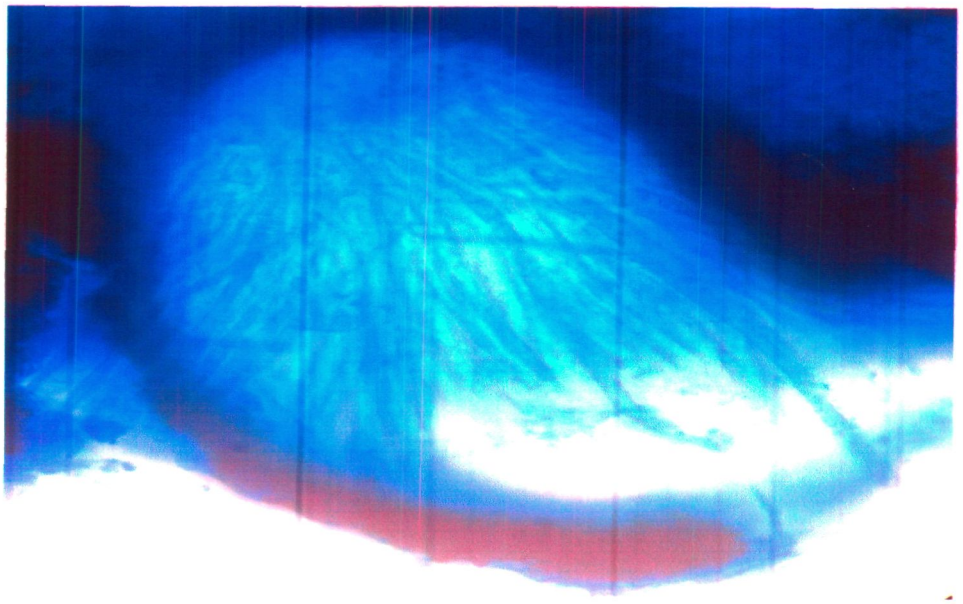

Fig: 52 An ascus showing ascospore (T.S).

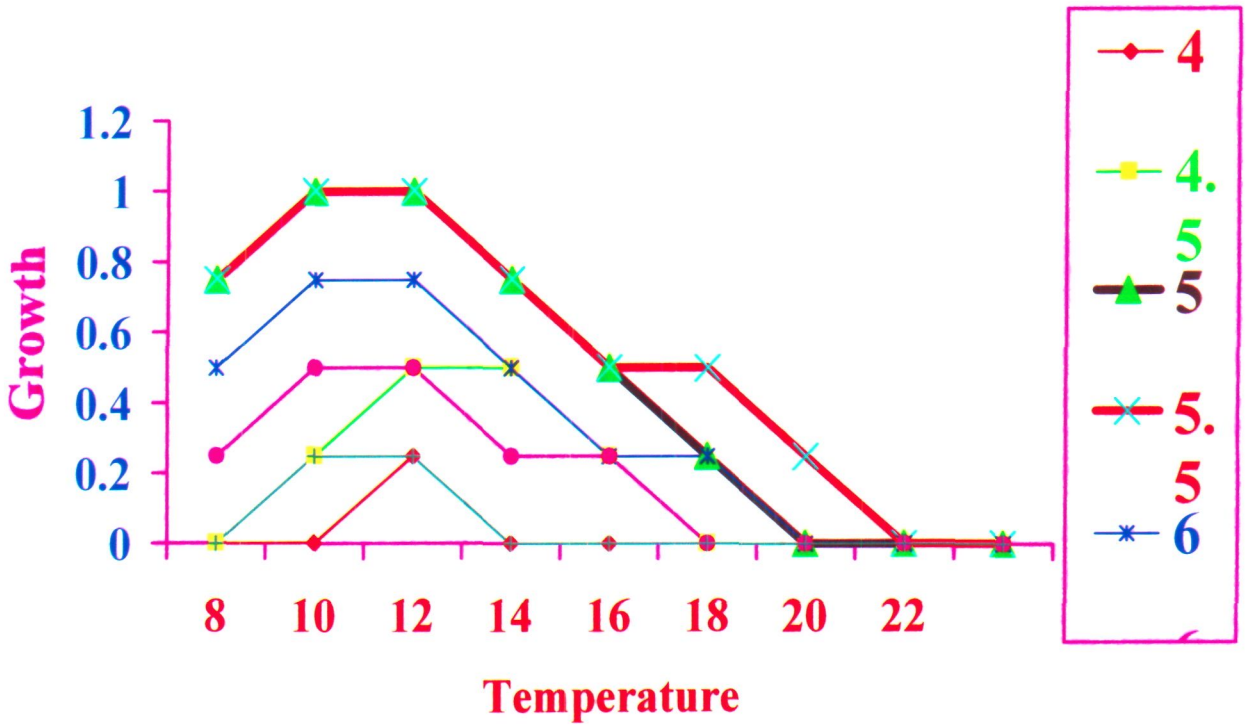

Fig: 53 Interaction of pH and temperature on growth of *Cordyceps sinensis*.

a. Growth on PDA

b. Growth on BEDA

c. Growth on SEDA

d. Growth on REDA

e. Growth on CHDA

Fig: 54 Successful *in vitro* culture of *Cordyceps sinensis* on artificial medium under laboratory conditions.

Fig: 55 Villagers being briefed about collection of Yartsa Gumba by Sh. K. S. Tolia, Block Pramukh, Munsiyari Block.

Fig: 56 Sh. K.S. Tolia Block Pramukh, Munsiyari with villagers on the way of collection of Yartsa Gumba.